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VOLUME IX JUNE, 1922 No. 2

BIONOMICS OF WEEVILS OF THE GENUS *SITONA*[1] INJURIOUS TO LEGUMINOUS CROPS IN BRITAIN

BY DOROTHY J. JACKSON, F.E.S.

PART II.

SITONA HISPIDULA F., *S. SULCIFRONS* THUN AND *S. CRINITA* HERBST.

(With 5 Text-figures and Plate III.)

A. *SITONA HISPIDULA* F.

SITONA HISPIDULA F. is widely distributed throughout Europe and America and is a recognised pest of leguminous crops in both continents. It is also recorded by Allard (1) from Western Siberia. In America its depredations appear to be only of recent date, as prior to 1876 this species was not known to occur, but in that year it was observed in New Jersey, and in 1889 its sudden spread in America was noted by Schwarz (15). It has since extended westward, and its first appearance in California was recorded by Van Dyke (20) in 1917.

In the British Isles it is common and widely distributed, and wherever present, is injurious to clover and lucerne, though the damage caused by it in this country has hitherto escaped recognition. The life-history of this species has been investigated by Wildermuth (22) in America, but in Europe only a few observations have been recorded by Brischke (4) in Western Prussia. Hitherto no account of the life-history of this species in Britain has been published.

Food-plants.

All species of clover (*Trifolium*), lucerne (*Medicago sativa*), medick (*Medicago lupulina*); rarely upon peas.

At Wye, Kent, this species was common upon lucerne in most months of the year; it also occurred on temporary clover leys, in fields of perman-

[1] The name *Sitona* Germ. is here adopted in place of *Sitones* Schoenh. on account of priority, Germar (9) having named this genus *Sitona* in 1824. Schoenherr (16) in 1826 and again in 1834 (17) also uses this name, but in 1840 (18) changed it to *Sitones*.

ent pasture and amongst clover in waste places in the same locality. Only one or two specimens occurred upon peas. In the north of Scotland it is locally abundant amongst clover in the fields and by the roadsides. In Russia and America this species is principally recorded as a pest of clover and lucerne, but in Maryland, Cory (5) mentions it as attacking newly planted Lima beans. Petit (12) observes that *S. hispidula* was so numerous upon lucerne in Michigan that entire fields were destroyed by it. Wildermuth (22) considers that the larvae of this species may sometimes feed upon the roots of grass, but no confirmation of this has been obtained in the present research. Bargagli (2) mentions the occurrence of *S. hispidula* on *Galega officinalis*.

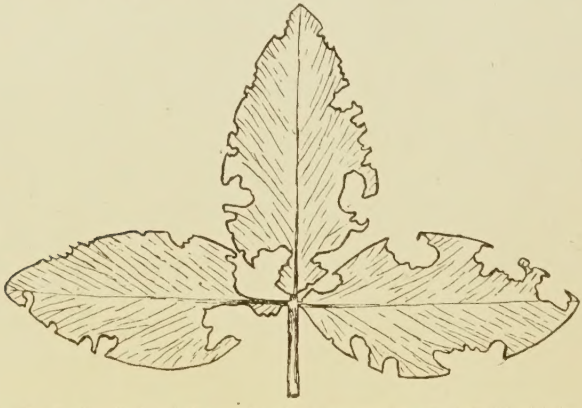

Fig. 1. Leaf of red clover showing damage by adult *S. hispidula*.

Nature of Damage.

Damage by adults. (Fig. 1.) The adults of *S. hispidula* feed upon the leaves of clover and lucerne but are rarely present in sufficient numbers to cause serious damage. They commence to feed at the edge of the leaf by biting out very small notches which are usually deepest between the veins, so that the eaten portion has often a jagged appearance. The beetles frequently continue to feed at the same place upon the leaf, thereby enlarging the original excavation and forming irregular indentations of various sizes.

Damage by larvae. (Figs. 2 and 3.) Though larvae of *Sitona* are to be found very commonly damaging roots of clover in the field it is not easy to determine to which species they belong, as at present there is no comparative description of the larvae published, and also when dug up from the field they are difficult to rear. The injury was therefore determined

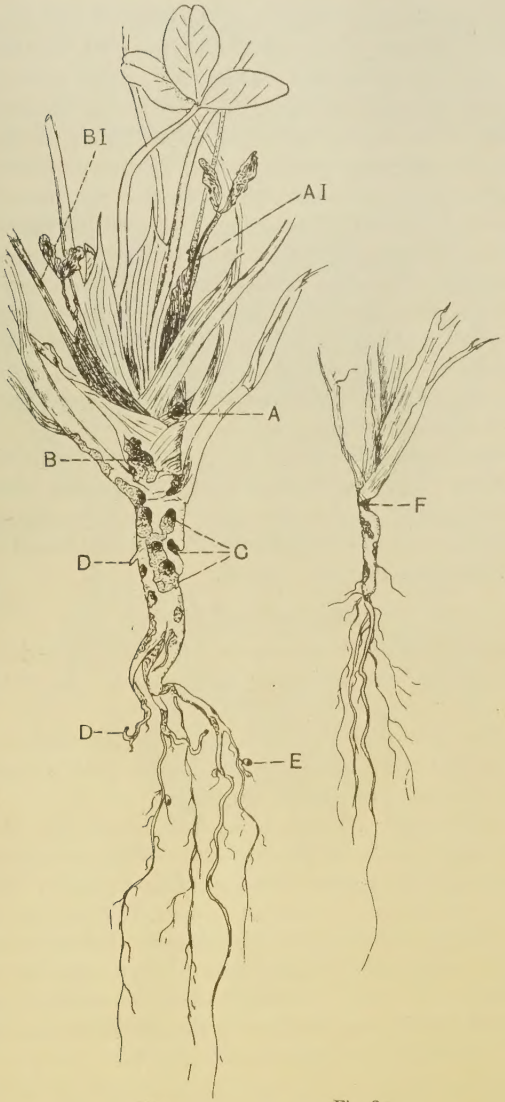

Fig. 2. Fig. 3.

Fig. 2. Plant of red clover with root damaged by larvae of *S. hispidula*. *A* and *B* = holes at base of plant bored by larvae and causing death of shoots *A* 1 and *B* 1. *C* = holes bored on root. *D* = side rootlets eaten by larvae. *E* = root nodule eaten by larva.

Fig. 3. Young plant of red clover almost bitten through at *F* by larvae of *S. hispidula*.

by breeding experiments. Eggs were introduced into pots of clover covered with muslin (10, p. 283, Plate XVIII, fig. B). In uninfected pots the clover remained strong and healthy, but in pots infected with eggs of *S. hispidula* much of the clover died before the larvae had ceased feeding owing to the damage they had inflicted upon the roots and the plants that survived were thin and weak. The larvae bored deep holes all over the main root and when half grown they were sometimes found entirely buried in the root. The portion of the root just below the crown of the plant was frequently chosen for attack with the result that the shoot immediately above the damaged area died. In all the larger plants so affected the outer shoots were dead from this reason whilst in small plants the whole root was sometimes bitten through at this point. The surface of the main roots were also gnawed in patches, the side rootlets were bitten off and the nodules destroyed. The gnawed portions of the root decayed and turned brown.

Field observations indicate that the greater part of the injury is done in June and July when the larvae are most abundant and plants of clover with the roots injured as described above have been dug up from the fields in these months and from the larvae that were found beside them adults of *S. hispidula* were duly reared.

Description of Adult.

Black, clothed on the dorsal surface with scales of various shades of brown and ochreous and with long erect setae on the elytra. Length 3·3–4·7 mm.

Head. Eyes flat, scarcely projecting from the sides of the head. Forehead between the eyes completely flat but with a narrow central furrow which is continued upon the rostrum.

Pronotum. Broader than long, sides strongly rounded. Covered with large diffuse punctures between which are smaller punctured dots, and bearing numerous short raised setae pointing forwards. Scales rather broad and closely placed, of uniform colour, but varying in different specimens; purplish brown or greyish brown. Raised setae black or white. Broad subdorsal bands and a narrow interrupted dorsal line composed of bright ochreous or whitish scales are usually present. The anterior coxal cavities separated from the presternal line by an area equal to the breadth of the presternum[1] (Fig. 5).

[1] Reitter (13) in his key to the genus *Sitona* makes use of the character afforded by the position of the anterior coxal cavities in regard to the transverse furrow behind the anterior edge of the prosternum. This furrow which he calls "die Abschnürungslinie hinter dem Vorderrande der Vorderbrust," I here designate as the presternal line and the area between it and the anterior margin as the presternum.

Elytra. Rather broad and short with striae of large punctures and with conspicuous raised setae, black and white, pointing backwards and arranged in lines. These setae are as long or slightly longer than the breadth of an interval between two striae. Scales much variegated in colour in the same specimen, occurring in darker and lighter groups.

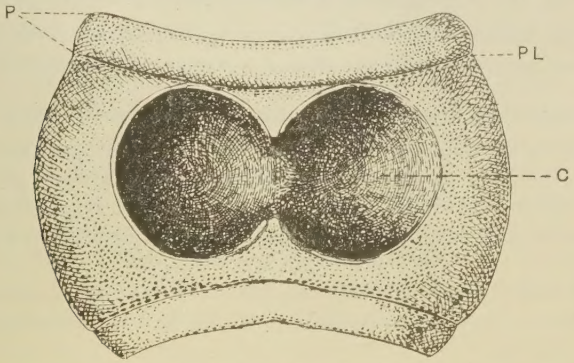

Fig. 4. Prosternum of *Sitona regensteinensis* Herbst with coxae removed. ×60. *P* = presternum. *PL* = presternal line. *C* = coxal cavity.

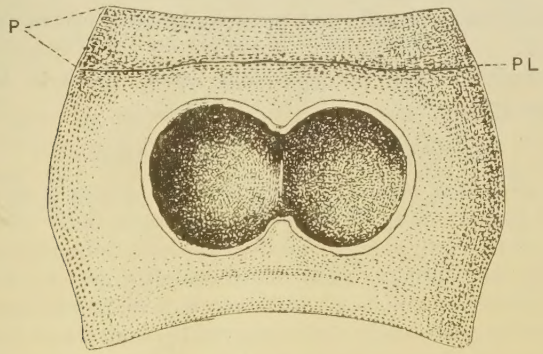

Fig. 5. Prosternum of *Sitona hispidula* F. with coxae removed. ×60. *P* = presternum, *PL* = presternal line.

Coloration varies in different specimens from dark purplish brown mottled with ochreous to pale greyish brown variegated with silvery grey. There is frequently a light patch upon the shoulders.

Undersurface. Clothed with ochreous or whitish scales and flat setae. The scales on the meso and metasternites and on the abdomen are plumate in structure.

Legs. Femora black but reddish at the base and extreme apex and bearing pale scales and long flat setae. Tibiae and tarsi red clothed with similar setae.

Antenae. Dark red with pale setae.

External Sexual Differences.

The sexes can readily be distinguished by examination of the posterior abdominal segments which are similar in structure to those of *S. lineata.*

On Distinguishing Sitona hispidula *from other British Species of* Sitona.

Owing to the difficulty usually experienced in identifying the weevils of this genus, and in order to supplement the key already given, the species which might most easily be confused with *S. hispidula* are here enumerated and some additional characters for their distinction are given.

S. tibialis Herbst and *S. lineella* Gyll. Bristles more depressed and much shorter than in *S. hispidula*, not being as long as breadth of an elytral interval.

S. crinita Herbst and *Waterhousei* Walt. Eyes prominent, projecting from the sides of the head.

S. regensteinensis Herbst. Anterior coxal cavities reaching presternal line (Fig. 4).

S. humeralis Steph. No upstanding setae. Forehead excavated between the eyes.

The Reproductive Organs of Sitona hispidula.

The reproductive organs of *S. hispidula* are similar in structure to those of *S. lineata*, but in the male differences occur in the shape of the genitalia. In the newly emerged female of *S. hispidula* the ovarian tubules are scarcely developed and the terminal chambers are very small, just as in the immature female of *S. lineata*, but unlike this species, the reproductive organs of both sexes attain full growth 6 to 8 weeks after emergence.

Alary Dimorphism[1].

In the course of dissection of *Sitona hispidula* F. it was observed that two forms of the species existed, one with fully developed wings (Plate III, fig. 1) and the other with very small vestigal wings of a peculiar shape (Figs. 2 and 3) and incapable of flight. The brachypterous wings vary in

[1] A similar case of alary dimorphism is described by Dr David Sharp in the Carabid *Pterostichus* (*Omaseus*) *minor* Gyll in *The Entomologist*, vol. 46, 82–87, 1913.

size and in distinctness of venation in different specimens, but no intermediate forms between Figs. 3 and 1 have yet been observed. The genitalia of the two forms have been examined and no differences have been detected, and moreover in captivity brachypterous males have mated with fully winged females.

In a separate article a description will be given of the structure of the two types of wings and of the modification of the metatergum in the brachypterous form.

Up to the present brachypterous specimens have only been taken in two localities, from Wye, Kent, and in Ross-shire. In the former district fully winged specimens predominated. In Ross-shire, around Evanton, only the brachypterous form has been found[1], but further north at Kildary fully winged specimens have also been taken. The macropterous form has been taken from the following localities: Crowborough, Sussex; Brandon, Suffolk; Tring, Herts.; Haslemere, Surrey; Kingussie, Inverness-shire; Invershin, Sutherland. The distribution of the two forms appears to have no relation to latitude or altitude, nor is the short winged type rare in localities where it occurs. Thus at Swordale, Evanton, about 500 feet high the latter form is abundant, yet at Invershin about 40 miles north near sea level, and at Balavil, Kingussie at an elevation of over 700 feet, *S. hispidula* is equally common, but all the specimens so far examined have been of the long-winged type.

The Egg.

The eggs vary slightly in shape and size from 0·41 mm. by 0·37 mm. or 0·46 mm. by 0·34 mm. to 0·49 mm. by 0·34 mm. They are similar in colour and shape to those of *S. lineata*. The first laid eggs of *S. hispidula* are pointed at both ends and twice as long as broad.

The Larva.

The larva closely resembles that of *S. lineata*, but the colour of the body, especially in the immature larva, is not as white as in that species, but more translucent and greyish. Slight differences occur also in the structure of the head by means of which it is possible to separate the larvae of the two species, and it is hoped to describe these fully in a later paper. No eye spots are present. The full grown larva measures about 6 mm.

The Pupa.

The pupa is similar to that of *S. lineata* and measures about 5 mm.

[1] Since writing the above a single ♀ of the fully winged form has been collected in this locality.

Life-History.

The life-history of most of the *Sitona* which breed upon clover is complicated by the long period of egg-laying of each female, with the result that the development of the progeny of the same parent extends over a considerable time. The life-history of *S. hispidula* may be thus summarised. There is only one generation in the year. The imagines lived about 12 months. They emerged from the pupal stage from July to September and commenced to lay eggs six to eight weeks after emergence. A few eggs were laid during the winter and vigorous oviposition recommenced in spring. Towards the end of June egg-laying decreased, and during July most of the weevils died. Eggs laid late in autumn did not hatch till the following spring, but a few of the September eggs and all those laid in spring and summer hatched in 25 days. No success was obtained in rearing the few larvae which hatched in autumn from the first laid eggs, but those which hatched in the following spring and summer fed up in from 11 to 14 weeks, pupated, and emerged as adults four weeks later. The last few eggs laid by the old females in July produced full fed larvae and pupae in the end of October, but these perished during the winter. Thus it will be seen that the principal period during which the larvae occur is in the summer from the end of April until August, those larvae which hatch before the winter from the first laid eggs and those which hatch late in the following summer from the last laid eggs of the same parents being few in number and uncertain in attaining maturity. The winter is thus passed almost entirely in the egg and imaginal state.

Detailed Observations on Life-History and Habits.

The life-history has been ascertained by field observations and breeding experiments. These may be placed in three groups according as they relate (1) to the imagines, (2) to the length of the egg stage, and (3) to the length of the larval and pupal period.

I. *The Imagines.*

Length of Life and Period of Oviposition.

(*a*) *Field Observations.*

Adult *S. hispidula* were obtained from various localities at different times of the year. If sexually mature the females laid eggs readily in the boxes in which they were collected. They were never subjected to artificial temperature. If immature, the beetles were sleeved in muslin bags upon

pots of clover kept out of doors and the date of oviposition observed. The results of these observations are here tabulated.

Date	Locality where collected	Condition	Remarks
1921 July 27th to Aug. 1st	Wye, Kent	Not laying eggs. Ovaries quite immature.	Fairly common.
1919 Aug. 11th to Sept. 1st	Bournemouth	Not laying eggs. Ovaries immature.	Sleeved on clover and commenced laying eggs from middle to end Sept.
1919 Sept. 4th to 8th	Sudbury, Suffolk		
1920 Sept. 16th	Inversbin, Sutherland	Some laying eggs and others not, ovaries of some mature, of others immature.	Very common.
1921 Sept. 20th	Evanton, Ross-shire		
1918 Oct.	Wye	Laying eggs.	Common.
1921 Jan. 31st and Feb. 5th	Evanton	Laying a few eggs.	
1921 Mar. 13th	Haslemere, Surrey	Laying eggs.	Common.
1918 1919 April and May	Wye	,, ,,	,,
1921 April and May	Evanton	,, ,,	,,
1918 June	Wye	,, ,,	Scarce.
1921 June	Evanton	,, ,,	,,
1919 July 30th	Sudbury	Laying a few eggs. Ovaries typical of very old female.	Only one old female obtained, easily distinguishable from the new generation by condition of reproductive organs.

(*b*) *Experiments.*

In order to determine the length of life and the period of oviposition adults collected at Wye in October 1918 and laying eggs were sleeved on clover growing in pots out of doors. On January 27th, during deep snow, some of the beetles were removed to a glass dish which was left in one of the pots, and two or three eggs were laid in it, but many of the beetles died, doubtless owing to insufficient protection from the severe frost. During the winter many of the remaining weevils in the pots died, but those that survived laid eggs in April and throughout May. By the end of May some had ceased oviposition and by June 22nd only a few individuals were still laying eggs, and still fewer in the beginning of July. By the middle of the month most of them had died, though one or two survived till August.

Period of emergence of imagines. This was determined by breeding experiments to be described later in which the weevils emerged during August and September. When sleeved on clover they commenced oviposition on October 22nd, about a month later than the specimens in

the field, but similar experiments tend to show that *Sitona* take longer to develop when bred in captivity.

Habits.

During fine weather in September and October the beetles are most abundant and may be obtained by sweeping clover or lucerne. They are very active on sunny days at this time of the year and are frequently to be met with on palings or stone walls. It is probable that the principal migration to the new fields occurs at this time. At Invershin, Sutherland, from September 16th to 21st, 1920, several hundred beetles of this species were observed upon the walls of a wooden building adjoining fields of grass and hay. On sunny afternoons numbers were seen crawling up from the ground, but despite careful watching I failed to observe any specimens flying on to the wall, although all the specimens examined from this locality proved to have fully developed wings. The weevils dropped down from the woodwork at the slightest touch. They were equally common on the walls in the shade as in the sun, but were rare on the stonework of the house. Many got in at the windows. *Sitona sulcifrons*, a very common species with brachypterous wings also occurred upon the walls but in much fewer numbers. The following year on revisiting this district on a fine day on September 21st no such swarms were observed, though the weevils were very common on the clover. This activity of the imagines in autumn is not confined to the winged individuals, but has also been observed in brachypterous specimens at Swordale, Ross-shire. In France, Bedel(3) has made some interesting observations upon a migration of *Sitona gemellata* in the end of September and beginning of October.

In the winter the adults of the *S. hispidula* continue to feed, and even during continuous frost in January and February, they were to be found in the fields in Ross-shire lying on the surface of the ground beneath freshly eaten clover leaves. On sunny days in March and April the beetles may be seen walking on the clover leaves but are more frequently taken at the base of the plant. They are also active at night. They lay their eggs indiscriminately wherever they happen to be resting.

Number of eggs laid. The number of eggs laid by a single female from the commencement to the end of oviposition has not been ascertained, but the following experiments carried out in the laboratory show the number of eggs laid by two females after hibernation. Artificial conditions are doubtless responsible for the greatly prolonged life of the female in the first experiment. This has happened with other *Sitona*

kept indoors and regularly fed, and no corroboration of such a prolonged life has been obtained by field observation or outdoor experiments.

		Apr. 14th to 30th 1919	May	June	July	Aug.	Sept. and Oct.	Nov.	Dec. to end Apr. 1920	May and June 1920	Total
1st ♀	No. of eggs laid	43	193	171	159	106	120	27	27	77	924
2nd ♀	„ „	63	148	Oviposition ceased							211

As a rule from two to five eggs are laid daily, but as many as twelve per day have been observed.

II. *Length of the Egg Stage.*

In order to determine the length of the egg stage, eggs laid between certain dates were placed on damp earth in small dishes and the latter kept in tins containing a layer of wet moss, the tins being kept out of doors or in an unheated room. The result of these observations may be tabulated as follows:

Locality from which parent beetles were obtained	Date of oviposition	Date of hatching
Bournemouth	Sept. 17th to Oct. 8	A few larvae only on Oct. 12th.
Invershin	Sept. 22nd to Oct. 13	Oct. 31st to Nov. 2nd three larvae only. Feb. 16th to April 25th, 42 larvae, the majority hatching in March.
Wye	Oct. 13th to 16th	Two larvae only on Nov. 18th.
Sudbury	Oct. 16th to 31st	Mar. 27th to Apr. 12th.
Evanton	May 6th to 10th	Commenced May 31st.

III. *Length of Larval and Pupal Stages.*

The time occupied in the development of larvae resulting from eggs laid at different times of the year has been ascertained by means of the following breeding experiments, in which, except when otherwise stated, the eggs were placed at the roots of clover growing in sleeved pots out of doors. It was necessary to duplicate many of these experiments to observe the habits and development of the larvae, owing to the disturbance of the soil this involved. The length of the egg stage being already known and the pupal stage lasting about four weeks the length of the larval stage can be deduced when the imagines emerge by subtraction of these periods.

1. *Development of the Autumn-laid eggs.*

(*a*) *The Autumn Hatching Larvae.*

Eggs laid September 17th to October 8th, 1919 produced a few newly-hatched larvae on October 12th. Eggs and larvae placed in pot on

October 12th. *December 5th*—soil of pot thoroughly searched but no larvae found.

(*b*) *The Spring Hatching Larvae.*

Eggs laid	Results
Sept. 17th to Oct. 18th, 1919	*Aug.* 31*st*, 1920. Imagines emerged.
Oct. and Nov., 1919	*June* 2*nd*, 1920. Larvae 2·2–3·3 mm. long occurring 1 or 2 inches below the surface soil, close to the small fibrous roots, and destroying the root nodules.
,, ,,	*July* 3*rd*, 1920. Larvae half to full grown. Injuring main root of clover.
,, ,,	*July, end.* Pupae.
,, ,,	*Aug.* 19*th*. Imagines.
	Larval period. 15–16 weeks.

2. *Development of the Spring and Summer Laid Eggs.*

Eggs laid	Results
Apr. 14th to 24th, 1919[1]	*June* 14*th*. Larvae 1·6 mm. long to 2·7 mm.
,, ,,	*July* 9*th*. Larvae 2·5–3·5 mm.
,, ,,	*July* 28*th*. Fully grown larvae.
,, ,,	*Aug.* 12*th*. Pupae.
,, ,,	*Aug.* 26*th to Sept.* 1*st*. Imagines.
	Larval period. About 11 weeks.
May, 1919	*Sept.* 17*th*. Imagines.
June 21st to July 21st[1]	*Oct.* 31*st*. Full grown larvae. Pupae.
	Nov. 28*th*. Pupae. Failed to rear adults.

The above experiments have been carried out in Ross-shire. It is probable that in the south of England the larvae develop more rapidly, as in fields at Wye, Kent, full grown larvae and pupae of this species were found on June 15th, from which imagines were reared from the beginning to the 21st July. From the field in Ross-shire pupae were obtained from July 5th to August 11th and adults reared from them from the end of July to the end of August. It was noticed that when larvae were reared in Ross-shire in a glass-house, the temperature being raised by the sun alone, the larval period occupied only eight weeks. During the winter and early spring repeated search was made for larvae of this species both in the south of England and the north of Scotland in localities where the adult was common, but always without success; *Sitona puncticollis* and *S. flavescens* being the only species found in the larval stage at that time.

Life-History in America.

In America, according to Wildermuth (22) the life cycle of this species occupies a very much shorter time. Thus the egg stage lasts 13 days, the

[1] These eggs were placed at the roots of clover previously planted out of doors in one of the large breeding cages already described (10, pp. 283–284, Plate XIX).

larval period 17–21 days and the pupal stage 8–10 days. Only one generation has been observed in the year.

NATURAL ENEMIES.

In America (6, 11) many birds have been observed feeding on the adults.

Insect Parasites.

No insect parasites have hitherto been recorded from *Sitona hispidula*, but in the course of this research three Braconids[1], *Perilitus rutilus* Nees, *Perilitus aethiops* Nees and *Pygostolus falcatus* Nees (the dark variety described by Ruthe) have been bred from the adult beetles. Single Hymenopterous larvae have been found on several occasions within the body of the beetles.

Protozoan Parasites.

Gregarines have frequently been observed in the alimentary canal of adult *Sitona hispidula* and also of *S. punctilollis*. Dr H. M. Woodcock has most kindly examined those from the latter species and has identified them as belonging to the genus *Gregarina*. Those from *S. hispidula* appear to be the same.

Fungus Parasite.

The fungus, *Botrytis bassiana* (Balsamo) Montagne appears to be the most serious natural enemy of this species and attacks both adults and larvae.

B. *SITONA SULCIFRONS* THUN.

Sitona sulcifrons is recorded by Reitter (13) as occurring throughout Europe and in the Caucasus and its injuries to various leguminous crops have been observed in France, Germany and Russia. In the British Isles it is widely distributed and is often exceedingly common upon red clover, especially in the north of Scotland. So far as I am aware, no observations have been recorded regarding the life-history of this species or the habits of the larvae.

Food-plants.

All species of clover (*Trifolium*) also bird's-foot trefoil, *Lotus corniculatus*. In France, Girard (24) records this species as damaging peas, and Allard (1) mentions its abundance on lucerne. According to Rushkovsky (26) peas, clover, lucerne and buckwheat are attacked by it in Russia. Rye (27) records the abundance of this species on lucerne on the south coast of England, but this I have not yet been able to corroborate.

[1] I am much indebted to Mr G. T. Lyle for his identification of these and other Braconids mentioned in this paper.

Nature of Damage.

Damage by Adult. The adults of *Sitona sulcifrons* feed upon the leaves of clover in the same way as those of *Sitona hispidula*. As a rule, however, the eaten areas are more regular than in that species and more or less U-shaped. From July to October nearly every clover leaf in certain fields of first and second year "seeds" in Ross-shire showed the characteristic notches eaten by this species, but the damage was never sufficient to check the growth of the plant. The adults could often be swept from the clover in numbers in this locality and out-numbered those of any other species of *Sitona*. In Kent, *S. sulcifrons* appears to be less generally distributed, but was abundant in temporary clover lays on the Downs at Wye, though rare at a lower elevation.

Damage by larvae. The larvae appear to feed principally upon the root nodules of the clover and they sometimes damage the small fibrous roots which bear them. Unlike the larvae of *S. hispidula* they have never been observed attacking the main root. The larvae occur in the soil to a depth of about 2 inches.

Description of Adult.

Black, sparingly covered with copper coloured scales and flat setae which are frequently abraded. Size 2·9 to 4·2 mm.

Head. Eyes prominent projecting from the sides of the head and with their dorsal edge higher than the level of the central furrow which runs down the middle of the forehead to the rostrum. The forehead between the eyes is not flat but gradually slopes downwards from the eyes on each side to meet the central furrow. Punctuation and scales very similar to pronotum.

Pronotum. Broader than long, covered with fairly closely placed punctures which, though comparatively large, are shallow. Sparingly clothed with flat copper coloured or ochreous setae, resembling scales but hair-like in width, and with indications of lighter dorsal and subdorsal lines composed of similar but more closely placed setae interspersed with elongated scales of pale yellow or copper. Anterior coxal cavities just reaching presternal line.

Elytra. Rather broad and short. Punctured striae most conspicuous anteriorly but becoming obsolete towards the apex. Individual punctures comparatively large. Intervals with finely punctured dots. Sparingly covered with elongated, usually copper coloured scales interspersed, especially on the sides, with flat setae of the same colour. Pale yellow or silvery scales occur in groups producing a variegated effect.

Sides and under-surface. A broad stripe of large pale scales extends along the sides of the thoracic segments commencing behind the eyes. The scales on the posterior portion of this band and also those on the ventral surface of the thorax are plumate in structure. Abdominal sternites covered with long whitish flat setae and a few plumate scales.

Legs. Femora black with pale flat setae and a few scales; tibiae and tarsi light red with similar setae.

Antennae. Light ferruginous, with pale setae, club darker.

External Sexual Differences.

The posterior abdominal segments differ in structure according to the sex as in *Sitona lineata.*

Species liable to be confused with S. sulcifrons *and characters which distinguish them.*

S. suturalis Steph. Eyes depressed and not projecting dorsally from the level of the forehead.

S. lineata L. Forehead, though with central furrow, quite flat between the eyes.

S. humeralis Steph., *S. puncticollis* Steph., *S. flavescens* Marsh and *S. cylindricollis* Fahraeus. Anterior coxal cavities not reaching presternal line.

S. sulcifrons is not likely to be confused with the bristle-bearing species of *Sitona.*

Wings of S. sulcifrons. (Plate III, Figs. 4 and 5.)

Specimens of *sulcifrons* collected from various parts of England and Scotland have proved on examination to have brachypterous wings (Fig. 5). These, however, are totally different in shape to those of *S. hispidula.* They are of nearly equal breadth throughout and are evenly rounded at the apex. They measure from 1·28 mm. long by 0·38 mm. broad to 1·44 mm. long by 0·49 mm. broad. The wings show even less trace of venation than those of *S. hispidula,* only a small portion of the costal and sub-costal nervures at the base of the wing being discernible. The wings are extremely delicate and often to be found folded irregularly into a narrow strip beneath the elytra.

A curious variation in the shape of the wings has been observed in a specimen collected at Invershin, Sutherland. In this (Fig. 4), the wing is very long and narrow, measuring 1·6 mm. long by 0·34 mm. broad and is narrowed towards the apex.

In relation to the vestigial character of the wings of *S. sulcifrons*, it is interesting to note that this species is often more abundant throughout the clover in first and second year "seeds" than any winged *Sitona* of the same habits, showing that impossibility of flight is no check to the local dispersal of the species. More information is required as to how much such winged species as *S. flavescens*, *puncticollis* and *hispidula* fly, and any observations on this point would be gratefully received. These species are very active upon their legs, but up to the present I have rarely observed any of them on the wing, and it seems probable that in many cases they may migrate to new crops in the same manner as *S. sulcifrons*.

The Egg.

The egg is similar to that of *S. lineata*. It varies slightly in size and shape from 0·37 mm. by 0·27 mm. to 0·41 mm. by 0·31 mm.

The Larva.

The larva is very like that of *S. lineata* and measures when full grown about 4·9 mm.

The Pupa.

The pupa measures from 3·2 mm. to 4·9 mm.

Life-History.

Sitona sulcifrons is a smaller species than *S. hispidula* and the egg, larval and pupal stages are all a little shorter. The reproductive organs of the adult also mature more quickly. Otherwise the life-history of this species much resembles that of *S. hispidula* and has been determined by similar breeding experiments and field observations which will be summarised as briefly as possible.

I. *The Imagines.*

Length of life and period of Oviposition. Newly emerged specimens, sexually immature, were taken in Suffolk in the end of July, and in Ross-shire on August 10th. Sleeved upon clover, oviposition commenced on September 12th and 25th respectively, and this was corroborated by field observations. Placed in pots of clover covered with muslin out of doors in Ross-shire, the majority survived the winter, and some lived until the following August. In the field, the beetles continue to feed during the winter, and even lay a few eggs. In April and May oviposition recommences vigorously, but few eggs are laid in June and still fewer in

July. In August, specimens of the old generation are rare in the field. The habits of the adults are similar to those of *S. hispidula*.

Emergence. In the breeding experiments the weevils emerged during August and September and commenced oviposition on September 17th.

II. *Length of Egg Stage.*

Locality from which parents were obtained	Date of oviposition	Date of hatching
Sudbury, Suffolk	Sept. 12th to Oct. 1st	Mar. 18th to 30th
Wye, Kent	Oct. 13th	Mar. 22nd to 27th
Evanton, Ross-shire	Nov. 19th to 27th	Apr. 2nd to 12th
Wye	Dec. 15th to 24th	Apr. 3rd to 24th
,,	May 27th to June 2nd	June 19th to 25th

From the above it will be seen that unlike *S. hispidula* none of the eggs laid in autumn by the newly emerged beetles hatched till the following spring.

III. *Length of Larval and Pupal Stages.*

The pupae occur in cells about $\frac{1}{2}$ inch below the surface of the earth. The pupal stage lasts about 24 days. The larvae developed as follows:

1. *From Autumn Laid Eggs.*

Locality where experiment was carried on	Date of oviposition	Results
In pots and large breeding cages in Ross-shire	Oct. and Nov., 1918	May 5th, 1919. Larvae 1·35 mm. long.
	,, ,,	June 30th. Some larvae full-grown, one pupa.
	,, ,,	July 17th to Aug. 25th. Emergence of imagines.
		Larval period. About 13 weeks.
In breeding cage at Wye, Kent	Oct. 13th to 30th, 1918	July 5. Full-grown larva and pupa.

2. *From Spring and Summer Laid Eggs.*

Locality	Date of oviposition	Results
Breeding cage, Ross-shire	Apr. 19th to May 6th, 1919	July 14th, 1919. Larvae 3–5 mm. long.
	,, ,, ,,	Aug. 19th. Full-grown larvae and pupae.
	,, ,, ,,	Aug. 23rd to beginning of Sept. Imagines.
		Larval period. About 11 weeks.
Pots, Ross-shire	May, 1919	July 28th, 1919. Small to half-grown larvae.
	,, ,,	Sept. 26th. Imagines.
,, ,,	June 6th to 13th	Nov. 23rd. Imago.
,, ,,	July 9th to 30th	Dec. 1st. 15 full-grown larvae, 10 of them dead. None survived.

In the above experiments larvae occurred from April to December. They were most common from June till the middle of August and at this

time they were abundant also at roots of clover in the fields in Ross-shire. The larvae obtained in the above breeding experiments in December died during the winter. It will be seen that they were the product of eggs laid in July and under natural conditions only a very few eggs are laid at this time. No larvae of this species have been found in the fields in winter though repeated search has been made in localities where the adult is common. A small *Sitona* pupa, probably of this species was, however, obtained on January 4th and it is possible that a few individuals, resulting from eggs laid late in the summer, may pass the winter in the pupal stage. Larvae of this species obtained from the fields in July gave rise to imagines from July 30th to October 1st.

Insect Parasites.

Insect parasites of *Sitona sulcifrons* appear to be rare and none have hitherto been recorded. Two Braconids, *Perilitus cerealium* Hal. and a species of *Liophron* have, however, been bred from adult *S. sulcifrons* and single Hymenopterous larvae have occasionally been found within the body of the beetles.

Fungus Parasite.

Similar to that of *S. hispidula*.

C. *SITONA CRINITA* Herbst.

Sitona crinita is one of the principal species mentioned by Miss Ormerod (25) and Curtis (21) as attacking peas and beans in England, and for this reason it has been included in the present research. So far, however, I have not found it sufficiently abundant on any crop to cause injury, but its profusion upon tares in the south of England is recorded by Walton (37) and Rye (27) and Mr S. R. Ashby tells me that he has found it very commonly upon vetches in Kent and in Cambridgeshire. At Wye, Kent, I have found it generally distributed and sometimes common on tares, but never abundant. It frequented the same food plants as *S. lineata* but was always vastly outnumbered by that species. It is rare in Scotland. Abroad it is widely distributed, occurring according to Allard (1), Reitter (13) and Henshaw (23), throughout Europe, in Central and East Asia, North Africa and America. It is recorded in Russia (24, 32, 36) as a pest of cultivated Leguminosae, and only in that country has its life-history been investigated (32).

Food-plants. Tares (*Vicia sativa*), lucerne (*Medicago sativa*), medick (*Medicago lupulina*), sainfoin[1] (*Onobrychis sativa*) all species of clover;

[1] Mr P. Harwood tells me he has taken *S. crinita* in abundance on sainfoin near Newbury in August, 1907.

less commonly upon peas and beans. Mr G. Fox-Wilson informs me that he found *Sitona crinita* seriously damaging the young flowers of *Cytisus biflorus* at Wisley on October 14, 1920.

Other recorded Food-plants. Rushkovsky (26) records this species from buckwheat in Russia, and Bainbridge Fletcher (30) from indigo and senji in India.

Nature of Damage.

The weevils eat semi-circular patches from the edges of the leaves. The larvae feed upon the root nodules and when nearly full grown they also occasionally bore channels in the main root close to the surface of the ground. The young larvae up to 2 mm. in length are to be found entirely buried in the root nodules, but when larger they feed freely upon them. An infected nodule can often be recognised by one end being darker owing to the excrement accumulated in it, and by the presence of a small hole through which the larva has entered.

Description of Adult.

Black, clothed with greyish white or ochreous scales and with raised setae on pronotum and elytra. Size 3·3 to 4·5 mm.

Head. Forehead broad, eyes very prominent. A central furrow commencing opposite the middle of the eyes is continued upon the rostrum and the area on either side of this furrow is slightly excavated. Unlike *Sitona Waterhousei* Walt., the breadth of the head across the eyes is barely twice the breadth of the rostrum at the apex. Pubescence and sculpturing as in pronotum.

Pronotum. A little broader than long, but much narrower than the elytra. With large deep closely placed punctures and evenly covered with broad ochreous or whitish scales and with short bristles. Narrow dorsal and broad sub-dorsal stripes are formed by lighter and more closely placed scales. Anterior coxal cavities separated from collar line by an area as broad as presternum.

Elytra. Shoulders prominent, sides almost parallel. With striae of medium-sized punctures; intervals minutely pitted. Scales similar to pronotum. Raised setae longer and backwardly directed, brown or white. Elytra frequently mottled with brown patches composed of long lineal brown scales; anteriorly often with indications of alternate darker and lighter longitudinal stripes (formed of darker and lighter scales) which may be continued to the apex of elytra.

Under-surface. Clothed with whitish ochreous plumate scales, and, on the abdomen, also with pale setae.

Legs. Femora black, those of 2nd and 3rd pair reddish at base and apex. Covered with pale scales and flat setae. Tibiae and tarsi ferruginous with pale setae.

Antennae. Rather short, ferruginous, with club darker.

External Sexual Differences.

Posterior abdominal segments similar in form to those of *S. lineata.*

Species liable to be confused with S. crinitus *and characters which distinguish them.*

S. Waterhousei Walt. Breadth of head across eyes 2½ times the width of the rostrum at the apex.

S. regensteinensis Herbst and *S. tibialis* Herbst. Anterior coxal cavities reaching presternal line.

S. lineellus Gyll. Forehead between the eyes quite flat.

S. hispidula F. See p. 98.

Wings of S. crinita.

All the specimens of *S. crinita* so far examined have had fully developed wings.

Eggs.

The egg measures from 0·34 by 0·26 mm. to 0·36 by 0·29 mm.

Larva.

The larva measures about 5 mm.

The Pupa.

The pupa measures from 3·8 to 4·9 mm.

Life-History.

The life-history of *S. crinita* is closely similar to that of *S. lineata* and can be summarised as follows:

Imagines. Newly emerged specimens, sexually immature, were obtained from peas in Suffolk on August 4th. Other specimens collected in September and October from lucerne, medick and seedling tares in Kent and Suffolk were also sexually immature. Sleeved upon clover out of doors these beetles laid no eggs till the following spring when oviposition commenced in May. Specimens collected in the field in April, May and June were laying eggs. The majority of the beetles collected from the field at this time died off before the autumn of the same year, but a few individuals have lived in captivity in the laboratory for over two years.

Length of egg and larval stages. Eggs laid on 4th June commenced hatching on June 26th. To determine the larval period eggs laid in the end of May were introduced into sleeved pots of beans. From these full-grown larvae and pupae were obtained on August 17th and the adults commenced to emerge from August 27th to September 19th. They laid no eggs till the following June.

Parasites.

A Braconid belonging to the genus *Perilitus* has been bred from an adult *Sitona crinita* and single Hymenopterous larvae have been found on several occasions within the body of these beetles.

The fungus *Botrytis bassiana* Balsamo (Montagne) attacks the adult beetles.

D. SUMMARY.

1. *Sitona hispidula* is common throughout Great Britain upon clover and lucerne.

2. The adults eat the leaves and the larvae damage the roots.

3. *Sitona sulcifrons* is frequently abundant upon "seeds" clover and the larvae feed upon the root nodules.

4. *Sitona crinita* frequents tares, clover, lucerne, etc., but is rarely sufficiently common to cause injury.

5. The adults of *S. hispidula* are either fully-winged or brachypterous and two forms of brachypterous wings have been observed in *S. sulcifrons*.

6. The life-history of these three species has been investigated in Britain for the first time.

7. There is only one generation in the year.

8. The adults live 12 months.

9. The period of oviposition and the length of the egg, larval and pupal stages varies according to the species.

10. The Braconids *Perilitus rutilus* Nees, *P. cerealium* Hal., *P. aethiops* Nees, *Pygostolus falcatus* Nees and a species of *Liophron* are recorded for the first time as parasites of the adult beetles.

11. The fungus *Botrytis bassiana* (Balsamo) Montagne attacks these species of *Sitona*.

REFERENCES.

Sitona hispidula F.

(1) *Allard, E. (1864). Séance du 23 Mars, *Ann. Soc. Ent. France*, iv, Series 4, 329–382.

(2) *Bargagli (1904). *Bull. Soc. Ent. Italy*, xxxvi, 8–10.

(3) Bedel, L. (1873). *Bull. Soc. Ent. France*, Séance de l'année 1873, cxcv.

(4) Brischke, G. S. A. (1876). *Entom. Monatsblätter* 1876, 38.

(5) Cory, E. N. (1915). Abstract in *Rev. App. Ent.* A. iii, 709.

(6) Dean, G. A. (1916). "Insects injurious to Alfalfa," *Kansas State Agric. Coll., Div. of Coll. Exten., Manhattan, Bull.* No. 5, 36 pp., 39 figs.

(7) Fabricius, J. C. (1776). *Genera Insectorum*, 226.

(8) *Fowler, W. W. (1891). *Coleoptera of the British Islands*, v, 216–226.

(9) Germar, E. F. (1824). *Insectorum Species Novae*, i, 414.

(10) Jackson, D. J. (1920). *Ann. of App. Biol.* vii, 2 and 3, 269–298.

(11) Kalmbach, E. R. and Gabrielson, I. N. (1921). Abstract in *Rev. App. Ent.* A. ix, 305.

(12) Petit, R. H. (1917). Abstract in *Rev. App. Ent.* A. vi, 340.

(13) *Reitter, E. (1903). *Bestimmungs-Tabellen der europäischen Coleopteran*, lii Heft Curculionidae, 9 Theil Genus *Sitona* Germ. and *Mesagroicus* Schönh.

(14) Reppert, R. R. (1921). Abstract in *Rev. App. Ent.* A. ix, 389.

(15) Schwarz, E. A. (1889). *Proc. Ent. Soc. Wash.* i, 248–249.

(16) Schoenherr, C. J. (1826). *Curculionidum Disposito Methodica*, 134.

(17) *—— (1834). *Genera et Species Curculionidum*, ii, Part 1, 96, 123 and 124.

(18) —— (1840). *Genera et Species Curculionidum*, vi, Part 1, 253.

(19) Stephens, J. F. (1831). *Illustrations of British Entomology*, iv, 134.

(20) Van Dyke, E. C. (1917). Abstract in *Rev. App. Ent.* A. v, 421.

(21) Webster, F. M. (1915). *U.S. Dept. Agr. Farmers' Bull.* 649, 1–8.

(22) Wildermuth, V. L. (1910). *U.S. Dept. Agr. Bur. Ent. Bull.* 85, Part 3, 29–38, figs. 15–19.

Sitona sulcifrons Thun.

(23) Bedel, L. (1873). *Bull. Soc. Ent. France*, Séance du 26 Mars 1873, l.

(24) Girard, M. (1880). *Bull. Soc. Ent. France*, Séance du 28 Juillet, 1880, xciii.

(25) Reitter, E. (1916). *Fauna Germanica, die Käfer des Deutschen Reiches*, v, 71.

(26) Rushkovsky, I. A. (1914). Abstract in *Rev. App. Ent.* A. iii, 481.

(27) Rye, E. C. (1865). *Ent. Monthly Mag.* i, 230.

(28) Thunberg, C. P. (1798). *Museum Naturalium Academiae Upsaliensis*, A. vi, 113, No. 4.

(29) Vassiliev, E. M. (1913). Abstract in *Rev. App. Ent.* A. i, 527.

S. crinita Herbst.

(30) Bainbridge, Fletcher (1917). *Report of the Proceedings of the Second Entomological Meeting, held at Pusa on 5th to 12th February*, 81 and 207.

(31) Curtis, J. (1883). *Farm Insects*, 347.

(32) Dobredeev, A. (1915). Abstract in *Rev. App. Ent.* A. iv, 139–140.

(33) Henshaw, S. (1885). *List of Coleoptera of America*, 136.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

D. J. Jackson del.

(34) *HERBST, F. F. W. (1795). *Natur system aller bekannten Insekten, Der Käfer*, Part 6, 245 and 354.

(35) ORMEROD, E. A. *Reports on Injurious Insects*, 1879, 8; 1880, 5, 6; 1881, 38, 39; 1883, 57–59; 1884, 3–5; 1886, 80, 81; 1889, 15–18; 1892, 102–116.

(36) SOPOTZKO, A. A. (1916). Abstract in *Rev. App. Ent.* A. IV, 293.

(37) *WALTON, J. (1846). *Ann. and Mag. Nat. Hist.* XVII, 234.

* The references marked with an asterisk refer to works in which other species dealt with in this paper are included, and they will therefore be omitted from subsequent bibliographies.

EXPLANATION OF PLATE III

Fig. 1. Fully developed wing of *Sitona hispidula.*

Fig. 2. Brachpterous wing of *S. hispidula.*

Fig. 3. A larger form of same with clearer venation.

Fig. 4. Wing of a specimen of *S. sulcifrons* from Invershin, Sutherland.

Fig. 5. Normal wing of *S. sulcifrons.* All magnified 40 times.

A = anal; *C* = costa; *CH* = head of costa; *CU* = cubitus; *FP* = flexor plate, or 3rd axillary; *R* = radius; *SC* = subcosta; *SP* = scapular plate, or 1st axillary; *X* = point of transverse folding of wing. (Nomenclature according to A. D. Hopkins in "The genus Dendroctonus," *U.S. Dept. Agr. Bur. Ent. Technical Series*, No. 17, Part I, 1909.)

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"SLEEPY DISEASE" OF THE TOMATO[1]

By W. F. BEWLEY, D.Sc.

(*Director of the Experimental and Research Station, Cheshunt, Herts.*)

(With Plates IV–VII.)

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I. INTRODUCTION.

Massee (14, 15) first described "Sleepy Disease" of tomatoes in Britain and attributed it to *Fusarium lycopersici* (Sacc.). In the present investigation it has been found that this nomenclature covers two discrete wilt diseases in this country. *Verticillium* wilt and *Fusarium* wilt. Sleepy Disease is found throughout the British and Channel Islands where tomatoes are grown and is responsible for considerable financial losses. *Verticillium* wilt occurs more frequently than *Fusarium* wilt, which is comparatively rare. In normal years the former appears about the middle of April and increases in intensity up to the second and third weeks in May. Usually the attacks die down during the second half of June, July and August and reappear at the end of September, when the plants die prematurely. *Fusarium* wilt occurs at the hottest part of the season, usually in July and August.

Plants attacked by *Verticillium* are usually stunted, while the internodes, especially the younger, are badly developed. When the conditions

[1] A grant in aid of publication has been received for this communication.

of temperature and light are favourable to the fungus, the disease symptoms appear quite suddenly and the plants wilt while still green. During the night the plants may recover their turgidity, only to wilt again as the morning advances. The leaves wither from the base of the plant upwards, adventitious roots emerge from the stem and the plant dies. Death is much slower when the conditions are less favourable to the fungus: yellow blotches appear on individual leaflets on the lower leaves and these leaflets wither.

II. ETIOLOGY.

1. The Causal Organism.

Since Massee described the disease as it existed in the British Isles in 1896, no further investigations have been carried out, and his views have been generally accepted in this country. He stated that the pathogen possessed two stages, the diplocladium and fusarium forms, produced from the same hyphae, but that only the fusarium stage was able to infect the plant. The present investigation is concerned with the disease of tomatoes grown under glass in the British Isles and especially in the Lea Valley. It has been found that the diplocladium and fusarium forms are not stages of the same fungus, but belong to different genera and each can, under definite conditions, produce a wilt.

In most cases *Verticillium albo-atrum* and various species of *Fusarium* may be found in the external growth at the base of a dead plant. A white growth of *Verticillium* first develops but soon becomes tinged with pink as it is overgrown by Fusaria. The almost constant appearance of *Fusarium* spores in this relation led to the idea that a species of *Fusarium* is always the cause of Sleepy Disease. During 1919–1920, 427 affected plants from different parts of England, Scotland and the Channel Isles were examined: 307 contained *Verticillium* alone, 77 contained *Verticillium* and either *Fusarium ferruginosum* or *F. sclerotioides*, 26 contained *Verticillium* and *F. oxysporum*, while 17 contained *F. lycopersici* alone. *Fusarium lycopersici* was the name given by Saccardo to a fungus which he found growing on decaying tomato fruits and has been universally applied to the species of *Fusarium* producing wilt disease of the tomato.

F. lycopersici is of comparatively little importance as a cause of tomato wilt in England, but is very destructive in America, where it is the primary cause of Sleepy Disease. Morphological and cultural studies have been made by Clayton (5), Edgerton (10), Wollenweber (20, 21) and others.

Reinke and Berthold (17) have figured and described the fungus *V. albo-atrum* as it occurs on the potato, and Carpenter (4) and Pethybridge (16) have more recently added certain details. The dominant spore form is unicellular but a considerable number of monoseptate spores are produced, especially in very old cultures, where indeed they may be the dominant form; and on certain media di- and tri-septate spores have been found.

The genus *Diplocladium* is distinguished from *Verticillium* only by having monoseptate spores, but in spite of the fact that monoseptate spores are produced in the present fungus, it is considered, that the many points of similarity to *V. albo-atrum*, in particular the fact that Reinke and Berthold originally described and figured monoseptate spores in their work, and secondly, the inoculation results obtained, entitle the *Verticillium* causing Sleepy Disease to be regarded as *V. albo-atrum*. Upon certain agar media, chiefly those to which asparagin has been added, slimy salmon-pink spore masses averaging 2 mm. by 1 mm. are produced resembling the pseudopionnotes of *Fusarium* cultures. The septate mycelium is hyaline at first but in most strains becomes brownish with age and varies from 2μ to 4μ in diameter. The mycelial cells, which give rise to microsclerotia, become swollen and by a process akin to budding a bead-like aggregate is formed, the cells of which thicken and turn brown. Radiating from the microsclerotial masses are strands of hyphae, unswollen, but thick-walled and brown. Strains which do not produce microsclerotia usually give rise to a small amount of this brown carbonised hyphae.

2. Inoculation Experiments.

Verticillium albo-atrum was isolated from wilted tomato plants in 1919 and tested for pathogenicity. Tomato plants, six weeks old, of the "Comet" variety were inoculated in various ways with a pure culture of the fungus. Six plants were used in each type of inoculation and six were left as controls. The stem was first washed with water, then mercuric chloride, alcohol and finally with sterile water. Small pieces of mycelium from a pure culture were pricked in and the wound covered with tinfoil. After inoculation all plants were placed in the tomato house. The controls to all experiments described in this paper were treated in precisely the same way as the inoculated plants except that no fungus was introduced.

1. *Stem Inoculations.*

(*a*) Hypocotyl. 27. iv. 19. Plants inoculated. 10. v. 19. Yellow blotches on leaf immediately above point of inoculation. 14. v. 19. First leaf wilted; second leaf

above with three yellow blotches on lamina. 21. v. 19. Bottom five leaves wilted; three lowest show yellow blotches; the other two wilted but green with "leaf roll." 4. vi. 19. Plants completely wilted. Controls healthy.

(*b*) Internodes between Cotyledons and first pair of leaves. 27. iv. 19. Plants inoculated. 12. v. 19. Yellow blotches on first leaf above point of inoculation. 14. v. 19. First leaf wilted. 17. v. 19. Second leaf above stab shows a yellow blotch. 20. v. 19. Three leaves on one side and one above the other wilted; fourth shows yellow blotch. 7. vi. 19. Leaves all round the plants are wilting. 11. vi. 19. Plants completely wilted. Controls healthy.

2. *Root Inoculation.*

27. iv. 19. Three of the biggest roots per plant inoculated. 20. v. 19. First pair of leaves show yellow blotches. 29. v. 19. Lowest four leaves wilted. 19. vi. 19. Plants completely wilted. Most leaves show "leaf roll" with very little yellowing. Control plants quite healthy.

3. *Soil Inoculations.*

Sterilised soil was inoculated copiously with spores from a pure culture and placed in 6″ pots, which were then planted with plants six weeks old. 27. iv. 19. Plants inserted in pots. 17. v. 19. First three leaves wilted. 10. vi. 19. Five plants completely wilted, the remaining one with only four top leaves healthy.

Inoculations were repeated at monthly intervals and the plants reacted to infection differently according to the time of year.

Table I.

Date inoculated. 1919	No. of days after inoculation that wilt occurred	Pathological symptoms
27th May	10	Complete wilt; no yellowing
27th June	No wilt in 70	Lowest 9 leaves turned yellow and partially dried up
25th July	,,	Lowest 3 leaves desiccated
25th Aug.	53	Complete desiccation of leaves from base upwards
22nd Sept.	40	Bottom 4 leaves desiccated, remainder wilted
22nd Oct.	26	Complete wilt with practically no yellowing

The preliminary experiments proved the pathogenicity of the *Verticillium* cultures employed, and indicated that the conditions during the months of June, July, August and September are unfavourable to the rapid progress of the fungus in the plants. The hypocotyl and internode inoculations were the first to show typical disease symptoms, but the soil inoculations, although longer in producing first symptoms, produced a complete wilt as soon as the hypocotyl inoculations. Internode inoculations were slower in producing a complete wilt. Here the fungus travelled up one side of the plant first and produced a wilt on this side only. After a time the fungus worked round the stem and induced a wilt

on all sides of the plant. During 1920 cultures were re-isolated from plants kept over the winter months and inoculation experiments performed to ascertain the effect of various environmental conditions upon the progress of the disease.

1920.

4. One re-isolated strain, V 33, was tested for pathogenicity and gave the first symptoms of the disease eight days after inoculation; complete wilt occurring one month after this. Further experiments were performed with this strain.

5. To ascertain the relation of the character of the plant to the progress of the disease, plants in different stages of growth and of varying degree of hardness and softness of growth were inoculated (*a*) by hypocotyl stab, (*b*) by planting in inoculated soil. The results are shown in Table II.

Table II.

Type of plant used	Mean diameter of hypocotyl in cm.	No. of plants	Age in weeks	Date of inoculation	Average no. of days between inoculation and appearance of first symptoms	Average no. of days between inoculation and total wilt
"Comet," soft growth	0·8	12	6	4. iv. 20	31	59
	1·0	12	8	4. iv. 20	21	47
	1·2	12	10	18. iv. 20	21	53
"Comet," hard growth	0·4	12	6	4. iv. 20	10	24
	0·7	12	8	4. iv. 20	8	29
	0·8	12	10	18. iv. 20	8	37
"Comet," starved growth	0·4	12	6	4. iv. 20	8	20

The observations shown in Table II indicate that plants grown hard with a thin stem, or plants obviously starved, most readily succumb to the pathogen in question. As there was very little difference in the result of the two kinds of inoculation, only those obtained by planting in infected soil are tabulated; these being the more comparable with those of plants naturally infected in the nurseries.

6. *Inoculation of sterile seedlings.*

The positive results obtained by growing young tomato plants in soil inoculated with a pure culture of *Verticillium*, does not eliminate the possibility that infection by this fungus may only be possible where the plant has been wounded previously. Under such conditions no obvious wounds may exist, but minute lesions may be present. Experiments

were therefore arranged in which seedlings grown under sterile conditions were inoculated. Tomato seeds were sterilised in mercuric chloride, washed in sterile water and germinated on agar in petri-dishes, after which they were transferred to 1000 c.c. Erlenmeyer flasks in which 200 c.c. Czapek's agar with 1 per cent. saccharose had been allowed to set. The seedlings were allowed five days in which to establish themselves in the flasks and then the medium was inoculated with a pure culture of *Verticillium*. The fungus readily attacked the young roots, which it penetrated and passing into the wood caused the seedlings to wilt, on an average nine days after inoculation. Sterile untreated controls were quite healthy after 20 days. From this it seems justifiable to assume that *Verticillium albo-atrum* can infect healthy tomato plants in the absence of wounds.

7. *Fusarium* inoculations.

Four species isolated from wilted tomatoes were tested, namely, *F. lycopersici*, *F. oxysporum*, *F. ferruginosum* and *F. sclerotioides*. Inoculations were performed upon plants six weeks old of the varieties Comet, Kondine Red, Fillbasket and Ailsa Craig. Plants in different conditions of health and under various conditions of temperature, humidity and light were inoculated. *F. ferruginosum* and *F. sclerotioides* never produced wilt under any circumstances and must be regarded as saprophytes. The strains of *F. oxysporum* destroyed the pith and cortical tissues round the point of inoculation and in some cases worked into the roots, destroying the tissues as they went. In a few cases, at an average temperature of 27·8° C. -28·9° C. a slight desiccation of the lower pair of leaves was observed, but generally no wilt or desiccation resulted from inoculation with this species. *F. lycopersici* readily produced a wilt and desiccation at temperatures of 28° C.–29° C., but when the temperature was below this, infection was uncertain.

3. Pathological Physiology.

The pathological symptoms in the anatomy of plants suffering from Wilt Disease, whether caused by *V. albo-atrum* or species of *Fusarium*, are limited to a brown discoloration of the wood vessels, and the presence of fungal hyphae within them. Early writers decided that the wilting or premature death by desiccation was due to the choking of the wood vessels with fungal hyphae and Pethybridge(16) suggested the term "Hadromycosis" for these symptoms instead of the older term "Vascularmycosis."

In studying *Verticillium* wilt, Van der Lek has suggested (19) that in this disease of the cucumber the fungal hyphae enter and kill the parenchymatous tissues of the leaves. Klebahn studying the *Verticillium* wilt of dahlia (12), Haskell the *Fusarium* wilt of potatoes (11), and Brandes the *Fusarium* wilt of banana (2) have all suggested the excretion by the fungus of a toxic substance, which is carried up the vascular bundles and kills the plant. This hypothesis gains a certain amount of support from the investigations by Dixon (7, 8) and Haskell (11) on the killing of plants by various artificial means.

In the present study it was found that the walls of the vessels are stained brown and in longitudinal sections a brown gum-like substance is frequently noticed lining the lumen and blocking up the vessels. In the process of staining this is washed away, but it is readily seen in fresh material. The similarity between the colour of this gum-like substance and that of the liquids in some old culture tubes, led to an examination of culture liquids. Cultures of *Verticillium* in Dox's solution with 20 per cent. saccharose showed a distinct yellow colour in the liquid. The fungus was therefore grown in this solution for 30 days, when the liquid, which was appreciably yellow, was filtered and used in the following experiments:

A. Erlenmeyer flasks were set up with 100 c.c. respectively, of the following solutions:

Series 1. Five flasks of filtrate from cultures.
„ 2. Five flasks of filtrate heated for five minutes at 100° C.
„ 3. Five flasks of sterile medium.
„ 4. Five flasks of sterile water.

Tomato seedlings 5 inches high were cut off under water near the roots, and placed one per flask in the above series. In series 1 the plants wilted in 17 minutes; in series 2 and 3 after 2 hours; while in series 4 the plants were perfectly turgid after 24 hours. As the plants in series 3 showed wilt in 2 hours, it was evident that exosmosis was taking place, so the experiment was repeated with the liquids diluted to three times their bulk with sterile water. In this case a definite wilt was produced in series 1 in 42 minutes and slight wilting in series 2 in 95 minutes which did not increase up to 24 hours. No wilt appeared in series 3 and 4 even after 24 hours.

B. To 1000 c.c. filtrate and 1000 c.c. sterile medium respectively, absolute alcohol was added. A cloudy precipitate formed and the solutions were allowed to stand until the precipitate settled to the bottom,

when the supernatant alcohol was syphoned off and the precipitate dried. After 24 hours the precipitates were dissolved in 3000 c.c. sterile water, and placed in flasks, 300 c.c. per flask.

Series 5. Five flasks with 300 c.c. each of solution of precipitate from culture filtrate.

„ 6. Five flasks with 300 c.c. each of solution as in series 5 heated for 5 minutes at 100° C.

„ 7. Five flasks with 300 c.c. each of solution of precipitate from the sterile medium.

Tomato seedlings severed from their roots were placed in these flasks as above described and a definite wilt was obtained in series 5 in 72 minutes. Series 6 yielded a slight wilt in 3 hours, but no wilt appeared in series 7 during 24 hours.

C. An attempt was made to isolate the exo- and endo-enzymes of *V. albo-atrum* by the technique devised by Brown (3), but difficulties arose in the process owing to the comparatively small size of the *Verticillium* spores. A much greater number of spores than was used by Brown had to be treated and the difficulty of obtaining a clean separation by centrifuging vitiated any attempt at quantitative determination.

The method adopted was as follows:

An abundant supply of spores was obtained by cultivating the fungus on Dox's agar with 1 per cent. saccharose, at 25° C. for 14 days, six petri-dishes of 6 inches diameter being used in each determination. The plates were covered by a thin layer of sterile water and the spores removed by gently scraping the fungal growth with a knife. Any rubbing with the finger as recommended by Brown for *Botrytis* had to be avoided, as such treatment rubbed the spores into the medium. The mixture of water, mycelium and spores was filtered through fine muslin and the filtrate consisting of water, spores and fine pieces of mycelium was centrifuged. It was almost impossible to separate much of the finer pieces of mycelium from the spores by this process, because of the lightness of the latter, but a good number of the larger pieces were removed. The spores were next germinated in strong turnip juice; 0·2 c.c. centrifuged spores being added to 21 c.c. turnip juice. Plates seven inches in diameter were each sown with 3 c.c. of the spore suspension, which was carefully spread over the surface. After 72 hours the mat of germinated spores was removed, and the turnip juice in which it had developed collected and tested for the presence of an exo-enzyme. The mat of mycelium was carefully washed to remove any spores, etc., and dried

over calcium chloride in vacuo. When dry, an equal weight of clean, dry quartz sand was added, and the material ground in a mortar. The dry material was extracted for 1 hour with sterile water, using 0·2 g. mixture to 3 c.c. water. The extract was tested for the presence of an endo-enzyme capable of producing wilt, but without success.

Exo-enzyme.

The turnip juice filtrate from the germinated spores was tested in two ways. In the first instance part was de-activated by raising the temperature to 100° C. and part untreated. Seedlings 6 inches high were cut off near the base under water and placed in the active and de-activated solutions. Others were also placed in the original turnip juice as controls. All liquids caused wilt in 20 minutes owing to their high osmotic pressure. They were diluted to three times their bulk with sterile water and the experiment repeated. The active solution caused wilt in 25 minutes, the de-activated solution in 105 minutes and the original turnip juice in 5 hours. Another portion of the original filtrate was treated with absolute alcohol until no further precipitation took place. The precipitate was allowed to settle, dried over-night, and taken up with sterile distilled water the next morning; part being left untreated and part de-activated at 100° C. Seedlings cut off at the base and seedlings with roots which had been thoroughly washed in running water were placed in the solutions. Wilt was produced in the cut seedlings but not in those with roots, the active solution producing a distinct wilt in 31 minutes, and the de-activated solution in 4 hours. Similar experiments were carried out with the extract from the ground mycelium, but no wilt was observed in any case.

The above experiments were repeated several times with precisely the same results. The wilted seedlings were sectioned and examined after 24 hours' treatment. Those wilted by the active solutions showed a browning of the wood for 5 cm. up the stem. Microscopical examination showed the presence of a brown gum in parts of the wood and near the end of the stem the cambium was destroyed. The seedlings in the de-activated solutions were soft at the end, but the wood was not browned; there was no gum, and no dissolution of the cambial layers.

The above results appear to warrant the assumption that under certain conditions a definite exo-enzyme is produced by *V. albo-atrum* capable of producing wilt. Such an enzyme may act directly in virtue of its function as an enzyme, or indirectly by reason of its gum-producing powers. It can be precipitated by absolute alcohol and dried. When re-

dissolved in water, it retains its power of producing wilt. Heating for 5 minutes at 100° C. greatly reduces its activity, but does not entirely de-activate it.

Enzyme production.

The wide range of artificial media, upon which the several strains of *V. albo-atrum* will grow indicates the probable secretion of a large number of enzymes. Modifying the methods elaborated by Crabill and Reed (6) the following specific enzymes were determined: amylase, inulase, emulsin, lipase, protease, erepsin and amidase. There was no good evidence of cytase production under the conditions tested.

4. Strains of *Verticillium*.

From April 27th to May 25th, 1920, over 50 single-spore isolations of *V. albo-atrum* were made. Small pieces of diseased wood were incubated in a moist chamber and spores transferred from the fungus growth which appeared to a drop of sterile water on a coverslip. Spore dilutions were made into other drops until each drop contained one or two spores. These drops were transferred to thin layers of potato agar in a petri-dish and the position of each spore marked after examination under the microscope. As soon as germination began the spores and young hyphae were transferred to sterile tubes of prune agar. The resulting isolations were classified into six groups varying in the rate, amount and kind of growth, and in the production of colour, carbonised hyphae and micro-sclerotia, when grown in Dox's solution with 1 per cent. saccharose. All the groups have been tested for pathogenicity and there is some indication that the virulence of the strains is related to the ability to produce carbonised hyphae and microsclerotia. Group I was uniformly slow in producing the characteristic wilt, while group VI was most rapid in its effect, as is shown in Table III.

5. Range of Hosts.

The host plants used were as follows: potato (*Solanum tuberosum*), egg plant (*Solanum melongena*), snapdragon (*Antirrhinum* sp.), cucumber (*Cucumis sativus*), sycamore (*Acer* sp.), cotton (*Gossypium herbaceum*), pepper plant (*Capsicum* sp.) and elm (*Ulmus* sp.). In the first four a definite Wilt Disease and subsequent desiccation was produced. In the sycamore and cotton the plants were much stunted and the leaves withered without wilting, but in the pepper the leaves wilted and remained green. The plants were stunted in the latter case, but only a few leaves were affected.

Table III.

Group no.	Isolation no.	Per cent. of microsclerotia produced as estimated in amount of surface of medium covered	Average no. of days from inoculation to appearance of first symptoms	Mean value	Average no. of days from inoculation to complete wilt	Mean value
I	2	0	31	31·3	72	65
	39		35		56	
	43		28		67	
II	2	10	24	25	54	55
	27		26		58	
	35		25		53	
III	4	30	22	24·6	46	48
	40		25		49	
	47		27		49	
IV	1	50	20	21·6	43	45
	22		23		47	
	45		22		45	
V	3	70	26	23·3	49	51·6
	13		22		54	
	30		22		52	
VI	5	100	17	16	42	40·3
	32		19		42	
	33		12		37	

In each case *V. albo-atrum* was isolated from the diseased plants. Even after four months there was no sign of yellowing or wilting of the inoculated elms, but they were shorter than the controls and *V. albo-atrum* was isolated from the wood 2 inches above the inoculation point.

6. Ecology.

Temperature.

The temperature relations of numerous fungi in pure culture have been studied in detail, but it is only within the last decade or so that the temperature factor has been related to the process of infection. This is largely the result of investigations carried out by the pathologists in the Bureau of Plant Industry in the United States Department of Agriculture and more particularly by Prof. L. R. Jones and his colleagues at the University of Wisconsin (18, 13, 9, etc.).

Such work is important especially in connection with the cultivation of crops under glass, where it is a simple matter to regulate the temperature. Observations made during the present investigation indicated an intimate relation between temperature conditions and inoculation results and showed the necessity for further inquiry into this relationship. As a series of glasshouses, where different temperatures could be maintained

constantly, was not available, inoculated plants (hypocotyl stab) were placed in certain positions in the experimental houses, corridors, etc. under as different average temperature conditions as could be arranged. Twelve plants were placed in each position, and the average temperatures were calculated from readings taken twice daily from maximum and minimum thermometers placed besides the plants. The final observations, shown in the following tables, were taken 21 days after inoculation, and where figures are given each represents the average of data obtained from 12 plants.

Table IV.

	Frame	Corridor	Tomato house	Cucumber house
Average temperature ° C.	**12·5**	**16·6**	**20·0**	**25**
Absolute minimum ° C.	5·6	11·1	12·2	20·6
Absolute maximum ° C.	20·6	22·2	27·8	33·3
Date of inoculation	14. iv. 20	14. iv. 20	14. iv. 20	14. iv. 20
No. of days after inoculation	21	21	21	21
Ratio of wilted to total leaves	**0 : 10**	**6 : 12**	**8 : 12**	**0 : 12**
Height of discoloured wood above stab	**15** cm.	**26** cm.	**28** cm.	**9** cm.
No. of days from inoculation to complete wilt	**49**	**28**	**28**	No wilt after **80** days

Table V.

		Tomato house		Cucumber house	
	Frame	Unshaded	Shaded	Unshaded	Shaded
Date of inoculation	14 iv. 20	14. iv. 20	14. iv. 20	14. iv. 20	14. iv. 20
No. of days from inoculation	21	21	21	21	21
Average temperature ° C.	**17**	**22**	**20**	**26·3**	**25**
Ratio of wilted to total leaves	**6 : 10**	**3 : 10**	**1 : 10**	**0 : 10**	**0 : 10**

While the results obtained are open to criticism because of the wide range of temperature to which the plants were submitted in any one position, certain empirical facts emerge which have been fully confirmed by observations in commercial nurseries. Chief among these is the beneficial effect which shade and temperatures above 24·0° C. have upon plants suffering from *Verticillium* wilt. Table IV shows that average temperatures of 16·6° C. and 20·0° C. are favourable to the rapid progress of the disease, that of 12·5° C. is unfavourable, while that of 25° C. practically inhibits it. It will be seen that the organism has travelled most rapidly up the stem, as indicated by the browning of the wood, at 16·6° C. and 20·0° C., and at these temperatures also complete wilt occurred most quickly. The results shown in Table V, while confirming the temperature relations, show the beneficial effect of shade. While

plants in the unshaded tomato house readily wilted, those in the shaded house exhibited only slight wilting. Observations in commercial glass-houses have shown that temperatures between 15·6° C. and 24·0° C. with an optimum of 21·1 to 22·8° C. are favourable to the rapid progress of *Verticillium* wilt. Below 15·6° C. and above 24·0° C. this is exceedingly slow, while suitable shading partially counteracts the effect of low temperatures.

A series of experiments was next arranged, in which wilted plants were transferred to conditions of high temperatures to ascertain if they would recover and if such recovery would continue, when the plants were returned to lower temperatures. The results are tabulated below.

Table VI.

No. of wilted plants	Length of time in shaded cucumber house (aver. temp. 25° C.)	Effect of high temperature	Length of time after returning to an aver. temp. of 20·0° C. before plants again wilted
12	1 day	Recovery	15 hours
12	2 days	„	15 „
12	7 „	„	2 days
12	14 „	„	3 „
12	30 „	„	16 „
12	75 „	„	30 „

Table VII.

No. of wilted plants	No. of days wilt has been visible prior to experiment	Percentage recovered in shaded cucumber house (aver. temp. 25° C.)	Percentage recovered in unshaded cucumber house (aver. temp. 25° C.)
20	2	100	100
20	7	100	100
20	14	100	100
20	21	100	90
20	30	100	80

The results in Table VI indicate that wilted plants recover when the average temperature is raised to 25° C. When such a temperature is operative for a short time, the effect is not a lasting one, for the plants rapidly wilt again when the temperature is lowered. Longer exposures to the higher temperature produce a more lasting result, for after 75 days at 25° C. the plants remained turgid for 30 days at a temperature favourable to wilt. In Table VII the percentage of wilted plants, which recover when transferred to a shaded house at an average temperature of 25° C., is compared with that of similar plants transferred to an unshaded house at the same temperature. Plants in different stages of wilt were used,

from a series where the wilt was just commencing to a series in an advanced stage after 30 days' wilting. All the plants recovered in the shaded house, but only a portion in that which was not shaded. The plants which did not recover in the unshaded house, being the badly wilted ones, were probably desiccated before they had a chance to recover. These observations appear to justify the conclusion that temperature is a most important factor in controlling the Verticillium Wilt Disease of tomatoes, while shading is valuable because it assists the plant, probably by reducing transpiration. The minimum, optimum and maximum temperatures for growth in pure culture of the strains of *Verticillium albo-atrum* utilised for the inoculations were 4·4° C., 23·3° C. and 30° C. respectively, and it will be seen that the optimum temperature for infection coincides approximately with the optimum temperature for growth in pure culture. *Verticillium* wilt is distinctly a disease of moderately low temperatures and is therefore most severe in the spring and autumn.

Soil factors.

Experiments carried out with different soils show that there is no obligate relation between *Verticillium* wilt and any particular soil type. Generally speaking, however, plants on soils which contain a large amount of humus show more disease than those growing on soils of a poorer nature. Clay soils, in virtue of their greater water-holding capacity, are cooler than sandy soils, and plants grown upon them are more prone to wilt than those grown on the latter.

III. CONTROL.

Investigations to determine the chemical agents best suited to eliminate the disease organisms from the soil and also to ascertain the effect of different manurial treatments upon the incidence of the disease are in progress and will be reported upon later.

1. Cultural methods.

Cultural methods for controlling the wilt disease have been devised and tested with promising results in the Lea Valley. In places where the disease has been common in previous seasons it is advisable to grow a highly resistant variety such as Manx Marvel or Bide's Recruit. Care should be taken to protect the plants from any check in their development and to encourage slightly soft rather than hard growth. When *Verticillium* wilt appears, the temperature should be raised until the average day and night temperature is above 25° C. This may be done

by suitably increasing the boiler heat, regulating the ventilation and closing the ventilators for two to four hours in the middle of the day. A light dressing of whitewash on the glass makes the conditions still more favourable for the plants. As little water as possible should be given to the roots as this aggravates the wilting, but a light overhead damping is beneficial. The development of fresh roots should be encouraged by mulching the base of the plant.

On one nursery 78 per cent. of the plants were showing symptoms of wilt disease before the above methods were enforced: a fortnight later only 10 per cent. remained wilted. In view of the fact that low spring temperatures favour infection by *Verticillium*, some advantage might be gained by planting later than is usually done, so that the higher summer temperatures may arrive before the plants are infected.

2. The elimination of sources of infection.

Edgerton (10) has pointed out that *F. lycopersici* develops much more rapidly in sterilised soil than in ordinary unsterilised soil. This has been found true for *V. albo-atrum*, for plants growing in imperfectly sterilised soil or re-inoculated sterilised soil yield a higher percentage of disease than those in unsterilised soil. This relation is accentuated if the soil be exceedingly rich in humus, as is the case with sterilised cucumber soil so frequently used for tomato propagation. The determination and elimination of infection thus becomes of vital importance. The fungal outgrowths at the base of dead diseased plants produce innumerable spores which become widely disseminated. These in themselves will not carry the fungus over the period of winter, but they readily germinate, and feeding on decaying plant material produce carbonised hyphae and microsclerotia, which are able to withstand winter conditions. Examination has been made of small pieces of plant remains, unearthed from nursery soils after the crop has been removed, and numerous tomato pathogens including *V. albo-atrum* have been found upon them. Thus it is important to remove completely all plants killed by wilt disease before cleaning up the nursery when the crop is finished and to remove carefully as much as possible of the general debris. The best way to remove the crop, when completed, is to sever each plant about 3 inches from the soil and remove all the aerial portions including leaves, etc., which have fallen to the ground, before attempting to remove the roots. If the surface is quite clean before the roots are removed, there is less chance of incorporating diseased material in the soil and the roots may then be carefully taken up, leaving behind only the very fine rootlets.

Another source of infection, which becomes more and more evident, is the contamination carried in "strikes" or baskets, and a considerable number of cases have been noted, where infection with various diseases has been traced to these articles. Baskets may be so mixed at the market that when they are returned, those from one nursery are sent to another, and so disease is spread. Baskets should not be taken near the growing plants for fear of introducing some new trouble, and during the winter months all baskets should be sterilised in readiness for the coming season. The importation of young plants from other nurseries is a procedure to be deprecated, for it is a fruitful means of disease dispersal. Contaminated water from surface wells is a constant source of infection with many diseases (1), and care should be taken to use a pure water supply. *V. albo-atrum* will infect a large number of cultivated plants, as well as certain trees, and while there is yet no direct evidence to show that the fungus may attack the common weeds around nurseries, it will probably be found that such is the case. It is desirable, therefore, that the immediate vicinity of nurseries be kept free from weeds, while potatoes and antirrhinums should not be permitted as they are susceptible to *Verticillium*.

In America, tomatoes resistant to the Fusarium Wilt Disease have been produced and an attempt is being made in this laboratory to raise a *Verticillium* resistant strain.

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SUMMARY.

1. "Sleepy Disease" or "Wilt" of tomatoes may be caused by one of two fungi, *Fusarium lycopersici* or *Verticillium albo-atrum*.

2. Massee's "diplocladium stage" of *F. lycopersici* has been shown to be *V. albo-atrum*.

3. The wilt producing fungi attack the roots and grow up through the vascular bundles into the stem, leaves and sometimes the fruits. The wood of a diseased plant is a light or dark brown colour.

4. The average temperature is a "limiting factor" in determining which fungus is active. *F. lycopersici* grows best at an average temperature of 27·8–28·9° C. If the temperature remains constantly much below this, little infection results. *V. albo-atrum* develops well at temperatures

from 15·6–24·0° C., being most active at 21·1–22·8° C. Above an average temperature of 25° C. little infection occurs.

5. The average temperature conditions existing in glasshouses in this country are generally too low for *F. lycopersici* and consequently it is rarely found as a cause of tomato wilt. The relatively low temperatures are favourable to *V. albo-atrum*, which accordingly is the most important cause of wilt.

6. Wilted plants soon die under conditions of low temperature, but if the average temperature be raised above 25° C., they recover and will bear a crop so long as the high temperature is maintained. When the temperature again drops, wilt reappears and death results.

7. *V. albo-atrum* from tomato readily induces wilt in the potato, egg-plant, snapdragon, cotton, pepper plant and cucumber, and produces a stunted condition of the sycamore and elm.

8. A number of different strains of *V. albo-atrum* have been isolated which vary in their rate of growth, the amount and rate of production of microsclerotia, and in colour production; but no evidence has been obtained to show that there may be different strains restricted to different varieties of tomatoes.

9. In pure culture the fungus has been shown to produce a large number of enzymes and there are strong indications that substances of a toxic nature play an important part in producing wilt.

10. There is a distinct relation between hardness of growth and susceptibility to wilt; the harder growing varieties and plants suffering from starvation or a severe check in the young stages being most susceptible to attack. Most varieties of tomatoes cultivated in this country are susceptible to *Verticillium*, but Manx Marvel has proved to be practically immune and Bides' Recruit highly resistant.

11. Certain cultural devices, including regulation of the temperature and shade, have been devised which assist "wilted plants" to recover.

12. Further investigations upon soil sterilisation and the production of resistant varieties are in progress.

APPENDIX.

Since this paper was written, a wilt disease of the sweet-pea has occurred in certain commercial nurseries where this crop is grown in the early part of the year before a tomato crop. The young seedlings showed first symptoms when about 6 inches high, the lower leaves turning yellow and withering. The desiccation advanced rapidly to the top of the seedlings, which then died. *Verticillium albo-atrum* was isolated and

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 1.

Fig. 2.

Fig. 1.

Fig. 2.

Fig. 1.

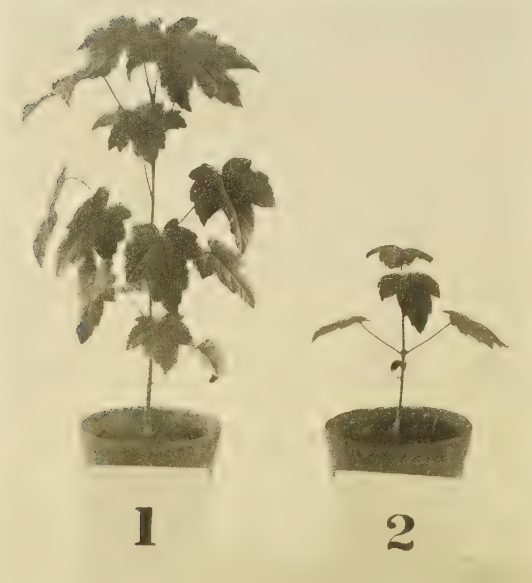

Fig. 2.

inoculation results proved it to be the cause of the disease. A series of cross-inoculations were undertaken with *V. albo-atrum* from the tomato, cucumber and sweet-pea, upon tomatoes, cucumbers and sweet-peas. In every case a definite wilt was produced and examination of the various isolations indicated that the fungi from these host plants are identical.

BIBLIOGRAPHY.

(1) Bewley, W. F. and Buddin, W. (1921). *Ann. App. Biol.* viii, No. 1.
(2) Brandes, E. W. (1919). *Phytopath.* ix, No. 9.
(3) Brown, W. (1915). *Ann. Bot.* xxix, No. 115.
(4) Carpenter, C. W. (1918). *Jour. Agr. Res.* xii, No. 9.
(5) Clayton, E. E. (1920). *Phytopath.* x, No. 1.
(6) Crabill, C. H. and Reed, H. S. (1915). *Biochem. Bull.* iv, No. 13.
(7) Dixon, H. H. (1914). *Proc. Roy. Dublin Soc.* xiv (N.S.).
(8) —— (1914). *Transpiration and the ascent of sap in plants.* Macmillan's Science Monographs.
(9) Edson, H. A. and Shapovalov, M. (1920). *Jour. Agr. Res.* xviii, No. 10.
(10) Edgerton, C. W. (1918). *Phytopath.* viii, No. 1.
(11) Haskell, R. J. (1919). *Phytopath.* ix, No. 6.
(12) Klebahn, H. (1913). *Mycol. Centr.* iii, Heft 2.
(13) Lauritzen, J. I. (1919). *Phytopath.* ix, No. 1.
(14) Massee, G. (1896). *Jour. Roy. Hort. Soc.* xix.
(15) —— (1910). "Diseases of cultivated plants and trees."
(16) Pethybridge, G. H. (1916). *Sci. Proc. Roy. Dublin Soc.* xv (N.S.), No. 7.
(17) Reinke, J. and Berthold, G. (1879). *Untersuch. Bot. Lab. Univ. Gottingen,* Heft 1.
(18) Tisdale, W. B. (1917). *Phytopath.* vii, No. 7.
(19) Van der Lek, H. A. A. (1918). *Over. u. d. Meded. v. d. Land.* D. xv.
(20) Wollenweber, H. W. (1913). *Ber. Deut. Bot. Gesell.* xxxi, Heft. 1.
(21) —— (1913). *Phytopath.* iii, No. 1.

EXPLANATION OF PLATES IV–VII

PLATE IV.

Fig. 1. Diseased tomato stem cut longitudinally to show the browned wood caused by *V. albo-atrum.*
Fig. 2. Old diseased tomato stem showing the fungal outgrowth at the base.
Fig. 3. Micro-photograph of *V. albo-atrum.*

PLATE V.

Fig. 1. (1) Wilted plant six weeks after inoculation with *V. albo-atrum.*
(2) Control plant.
Fig. 2. This photograph shows the wilted plant in Fig. 1 after being submitted to shade and an average temperature of 25° C. for 30 days. The wilted leaves have fallen off, but the plant has recovered and made good growth in the top.

Fig. 3. (1) Plant inoculated with *V. albo-atrum* at an average temperature of 20·0° C. (2) Control plant.

Fig. 4. (1) Recovery which resulted from placing the wilted plant (Fig. 3 (1)) under an average temperature of 25° C. for 24 hours. (3) Inoculated plant left at 20·0° C. showing wilt.

PLATE VI.

Fig. 1. Antirrhinum. (1) Control. (2) Inoculated with *V. albo-atrum* by a prick in the stem: wilt shows on one side only. (3) Plant grown in infected soil.

Fig. 2. Potato. (1) Control. (2) and (3) Inoculated with *V. albo-atrum.*

PLATE VII.

Fig. 1. Cotton plant. (1) Control. (2) Inoculated with *V. albo-atrum.*

Fig. 2. Sycamore. (1) Control. (2) Inoculated with *V. albo-atrum.*

(*Received Oct.* 18*th*, 1921)

BIOLOGICAL STUDIES OF *APHIS RUMICIS* LINN. REPRODUCTION ON VARIETIES OF *VICIA FABA*.

By J. DAVIDSON, D.Sc.

(*From the Entomological Department, Institute of Plant Pathology, Rothamsted Experimental Station, Harpenden.*)

WITH A STATISTICAL APPENDIX BY

R. A. FISHER, M.A.

(With 1 Text-figure.)

I. INTRODUCTION.

The following paper is a continuation of previous work(1) and gives results showing the varying reproductive capacity of *Aphis rumicis* on eighteen different varieties of field beans.

My sincere thanks are due to the following gentlemen who very kindly supplied me with seed for these experiments: Prof. J. Percival, M.A., University College, Reading; Mr R. M. Wilson, Principal, East Anglian Institute of Agriculture, Chelmsford: and The Director, Sveriges, Utsädesförening, Svalöf, Sweden.

My thanks are also due to Mr R. A. Fisher for his kindness and assistance with the statistical considerations involved in this paper.

The following abbreviations are used in the text: w. v. ♀ = winged viviparous female; a. v. ♀ = apterous viviparous female; 1st v. gen. = 1st, 2nd, etc. viviparous generations.

II. METHODS EMPLOYED.

The plants were grown in unmanured soil to which 10 per cent. of sand was added to prevent "caking" of the surface. Ordinary flower pots, 10 inches in diameter, were used. These were coated on the outside with a layer of wax in order to prevent excessive evaporation. The plants were kept covered with muslin bags throughout the experiments[1].

All the experiments were carried out under similar conditions in a large glasshouse specially designed with large windows and doors. The

[1] The amount of light cut out by these covers was estimated with a thermopile and found to be about 22 per cent. There was very little effect on the growth of the plant.

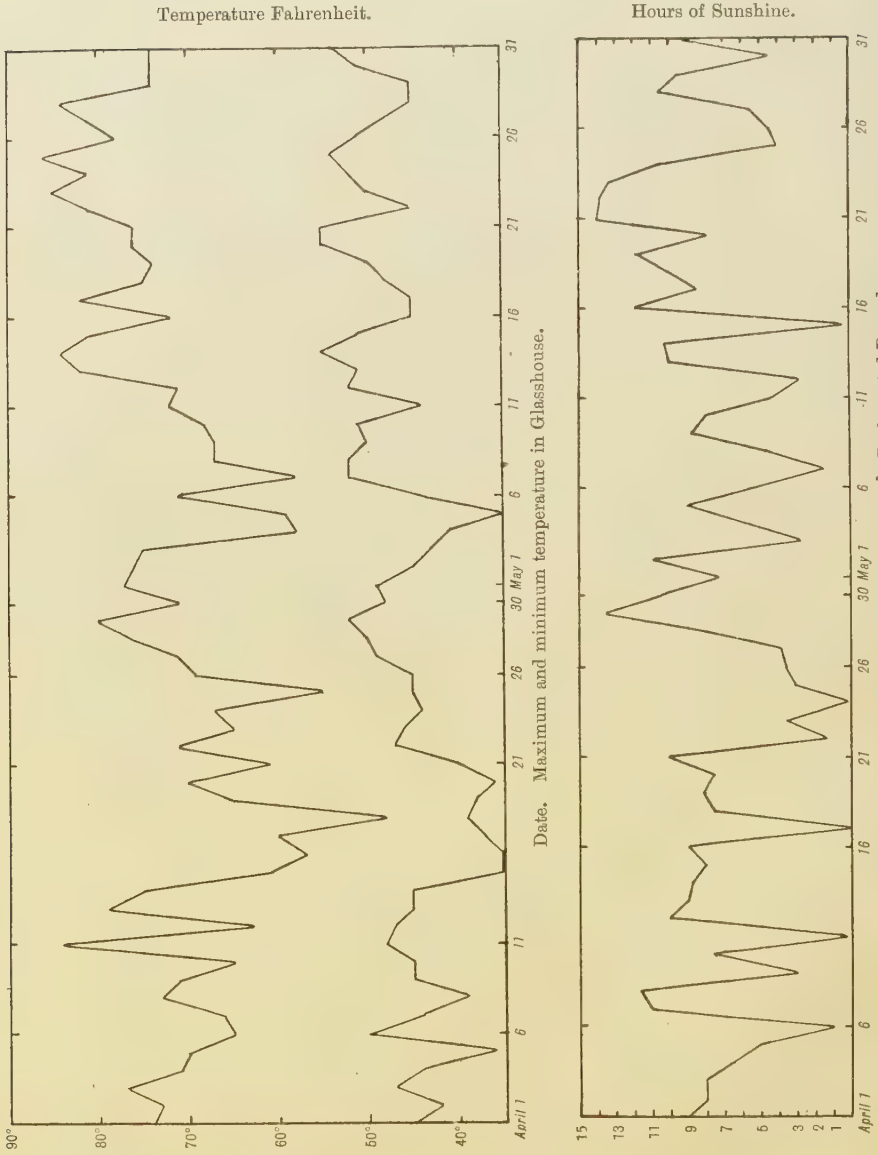
Temperature Fahrenheit.
90°
80°
70°
60°
50°
40°
April 1
6
11
16
21
26
30 May 1
6
11
16
21
26
31
Date. Maximum and minimum temperature in Glasshouse.
Hours of Sunshine.
15
13
11
9
7
5
3
2
1
April 1
6
11
16
21
26
30 May 1
6
11
16
21
26
31
Date. Hours of sunshine, April and May, 1921. Rothamsted Records.

Fig. 1.

maximum and minimum daily temperature in the glasshouse throughout the experiments is shown in Fig. 1.

There were six pots of each variety of field beans and six pots of garden Prolific Longpod broad beans. In all cases each pot contained a single plant. The latter variety of beans, which gave a high figure of infestation in the 1920 experiments, is taken as the standard to which the other varieties are referred in order to get relative figures of infestation. One plant of each variety was the "stock" plant for the variety, and the remaining five plants were utilized for the infestation tests.

The seeds were all sown on 25. iii. 21.

The aphids used in the experiments (*Aphis rumicis*) were derived from one egg, all being the offspring of one Fundatrix. Oviparous females, which developed in the colonies in the 1920 experiments, laid eggs in October 1920 on *Euonymus europaeus*. Some of the eggs commenced to hatch out on 8. iii. 21, and one Fundatrix was isolated on *Euonymus europaeus* on 25. iii. 21. This individual was the "stem-mother" of all the individuals used in the present experiments. As the individuals in each generation became adult they were isolated. The Fundatrix produced a. v. ♀♀ of 1st v. gen. These gave rise to a mixed progeny of w. v. ♀♀ and a. v. ♀♀ in the 2nd v. gen. Two winged migrants were transferred on 26. iv. 21 to each of the "stock" plants referred to above. On these they produced a. v. ♀♀ of 3rd v. gen. When these a. v. ♀♀ were almost adult and before they actually began to reproduce, the five plants of each variety were separately infected on 11. v. 21 with an a. v. ♀ derived from the stock plant of the variety concerned. The date and time when the a. v. ♀ on each plant began to reproduce was recorded (*vide* Tables) and reproduction was then allowed to go on for 14 complete days. The total number of aphids produced on each plant at the end of that period was then counted.

It will be noted that the infections of the different plants were made on the same day and that the 14-day reproduction period extended over practically the same period for all the varieties, thus ensuring that factors of temperature, humidity and sunshine were the same for all.

Further points which should be considered in experiments of this kind have already been given in the paper referred to above.

III. DISCUSSION.

Tables I and II shows the results obtained for the 18 varieties of field beans. These may be compared with the results obtained for Prolific Longpod (XIX) which is taken as the standard in order to fix the relative values of susceptibility of the other varieties.

Table I.

Variety	No. of variety	No. of plant	Date in May a. v. ♀ commenced producing	Date in May aphids killed off	Total aphids produced	Mean figure of infestation and probable error
Bohus beans (Sweden)	I	1	13	27	1275	
		2	,,	,,	1058	
		3	,,	,,	967	1018±51·3
		4	,,	,,	904	
		5	,,	,,	884	
		6		Stock plant		
Mazagan	II	7	12	26	1188	
		8	,,	,,	1154	
		9	,,	,,	1068	1007±51·1
		10	,,	,,	992	
		11	,,	,,	632	
		12		Stock plant		
English field	III	13	13	27	978	
		14	,,	,,	759	
		15	,,	,,	680	737±43·7
		16	,,	,,	636	
		17	,,	,,	631	
		18		Stock plant		
Heligoland	IV	19	14	28	738	
		20	16	29	723	
		21	14	28	622	616±39·9
		22	,,	,,	502	
		23	,,	,,	497	
		24		Stock plant		
Scotch var. Granton	V	25	13	27	753	
		26	,,	,,	677	
		27	,,	,,	544	591±39·1
		28	,,	,,	542	
		29	,,	,,	441	
		30		Stock plant		
Winter beans (Dorset)	VI	31	13	27	641	
		32	,,	,,	630	
		33	,,	,,	575	564±38·2
		34	12	26	542	
		35	13	27	430	
		36		Stock plant		
Winter beans (Somerset)	VII	37	13	27	656	
		38	,,	,,	525	
		39	,,	,,	501	513±36·4
		40	,,	,,	458	
		41	12	26	426	
		42		Stock plant		
Winter beans (Essex)	VIII	43	13	27	541	
		44	,,	,,	536	
		45	,,	,,	450	451±38·2
		46	,,	,,	276	
		47	,,	,,	—	
		48		Stock plant		
Spring tick (Suffolk)	IX	49	13	27	487	
		50	,,	,,	413	
		51	,,	,,	407	419±32·9
		52	,,	,,	396	
		53	,,	,,	391	
		54		Stock plant		
Carse beans	X	55	13	27	650	
		56	,,	,,	477	
		57	,,	,,	425	415±32·8
		58	14	28	349	
		59	13	27	176	
		60		Stock plant		

Table II.

Variety	No. of variety	No. of plant	Date in May a. v. ♀ commenced producing	Date in May aphids killed off	Total aphids produced	Mean figure of infestation and probable error
Kilbride beans	XI	61	13	27	522	
		62	,,	,,	446	
		63	,,	,,	441	423±33·1
		64	,,	,,	391	
		65	,,	,,	317	
		66		Stock plant		
Winter beans (Hertfordshire)	XII	67	13	27	593	
		68	,,	,,	461	
		69	,,	,,	384	404±32·4
		70	,,	,,	320	
		71	,,	,,	263	
		72		Stock plant		
Small tick	XIII	73	13	27	648	
		74	,,	,,	383	
		75	,,	,,	374	410±32·6
		76	,,	,,	335	
		77	,,	,,	312	
		78		Stock plant		
Winter beans (Berkshire)	XIV	79	13	27	468	
		80	,,	,,	406	
		81	,,	,,	388	371±34·7
		82	,,	,,	222	
		83	,,	,,	—	
		84		Stock plant		
Winter field (Cheshire)	XV	85	13	27	457	
		86	,,	,,	413	
		87	,,	,,	412	375±31·2
		88	,,	,,	332	
		89	,,	,,	263	
		90		Stock plant		
Scotch spring beans	XVI	91	13	27	495	
		92	,,	,,	465	
		93	,,	,,	363	391±31·8
		94	,,	,,	333	
		95	,,	,,	299	
		96		Stock plant		
Winter bean (Suffolk)	XVII	97	14	28	433	
		98	,,	,,	333	
		99	,,	,,	326	286±27·2
		100	,,	,,	184	
		101	,,	,,	156	
		102		Stock plant		
Vicia narbonensis	XVIII	103	14	28	129	
		104	15	29	33	
		105	,,	,,	9	37
		106	14	28	11	
		107	15	29	5	
		108		Stock plant		
Prolific Long-pod	XIX	109	13	27	1205	
		110	,,	,,	1147	
		111	,,	,,	1015	1037±51·8
		112	,,	,,	969	
		113	,,	,,	848	
		114		Stock plant		

The regular grouping of the figures showing the total number of aphids produced in 14 days (column 6) indicates that there is a significant difference in the degree of infestation of different varieties over a given period of time.

If we take the arithmetic mean of the total numbers produced on the five plants in each variety (column 7) we obtain for each variety a mean figure of infestation resulting from one apterous mother in a 14-day period. To this is attached its probable error calculated as explained in the Appendix.

The varieties therefore may be grouped into six distinctive classes, each class having a mean figure of infestation as shown in Table III. The mean figure of infestation for each class is taken as the arithmetic mean of all the means of the varieties included in the class.

Table III.

Class and mean figure of infestation	Prolific Longpod 1037	A 1012	B 737	C 570 ± 19·2	D 407 ± 11·1	E 286	F 37
Varieties	XIX	I	III	IV	VIII XII	XVII	XVIII
		II		V	IX XIII		
				VI	X XIV		
				VII	XI XV		
					XVI		
Degree of susceptibility	Standard variety taken as 100 %	98 %	71 %	55 %	39 %	27 %	3 %

Referring each class to the variety Prolific Longpod (XIX), the mean infestation figure for which is 1037, the relative degree of susceptibility of the varieties in each class may be expressed in percentages as shown in Table III.

Conversely by subtracting these percentages from 100, one gets the relative degree of resistance to infestation of the varieties in the different classes.

After 14 days reproduction on varieties in class A, the plants may be considered as fairly heavily infested, while the varieties in class D have only a moderate infestation and those in class E less so. Class F has almost a negligible infestation.

In 21 days the varieties in class A would be practically destroyed by the aphid infestation, those in classes D and E less so and in class F almost negligible.

There is no class here representing complete immunity from attack but the aphids were compelled to stay on these plants. In nature winged migrants would select their host and probably not reproduce on unfavourable varieties.

The classes evidently show significant differences in the degree of infestation in a given period.

It would appear probable that owing to the influence of the cell sap of the varieties concerned on the aphid metabolism, the rate of reproduction is considerably affected and thus under the same conditions of environment, the chances of infestation occurring are greater with some varieties than others.

This in itself is an important economic consideration in that, with the prospects of heavy rain or broken weather conditions (unsuitable for aphids) within a two or three weeks' period of fine weather, the chances of varieties in classes D, E and F recovering from aphis attack, are much greater than would be the case with varieties in class A.

Plant-breeding experiments would show whether susceptibility or resistance is a specific mendelian character. It seems feasible to consider tentatively, that the factor or factors (genes) which make for high resistance as in *Vicia narbonensis*—which as discussed below probably represents the prototype of *Vicia faba*—may have been present in the original wild bean, and that this character has been lost or modified in the process of selection in the cultivated varieties.

A theoretical discussion of this question of varying susceptibility would hardly be profitable at this stage. It would appear, however, that the factor or factors concerned are associated with the general physiology of the plant and have an influence on the cell sap.

If one factor only is concerned, it should not be a difficult matter to trace it by cross breeding experiments. On the other hand, if the character of resistance is due to an interworking of a number of factors, the problem becomes an extremely complex one.

It is interesting to consider variety XVIII (*Vicia narbonensis*) in this respect.

Some authorities consider this species as a prototype of the cultivated *Vicia faba*.

According to De Candolle (2) the bean has been cultivated from prehistoric times, and may have been distributed during the early migration of man. Some thousands of years ago it was probably established wild in two areas, namely, south of the Caspian sea and North Africa.

Vicia narbonensis most nearly represents the wild prototpye of the modern cultivated race. It is found wild to-day in the Mediterranean area; east towards the Caucasus; in Northern Persia and Mesopotamia[1].

[1] I have tried Prolific Longpod ♂ × *Vicia narbonensis* ♀ in 1920 and have reason to believe that three pods which came to maturity are successful crosses. The seeds from these pods are being carried to the F_2 generation.

The improved conditions associated with good cultivation, manurial treatment, etc., may to some extent influence the degree of susceptibility to aphis attacks. In the case of wild and cultivated varieties, the grouping of the 18 varieties used in these experiments (Table III) indicates that these factors are not the only considerations. The infestation figures obtained for the 10 varieties of broad beans experimented with in 1920 (*loc. cit.*) showed no significant difference in the degree of susceptibility.

IV. SUMMARY.

The reproduction of *Aphis rumicis* was tested on 18 varieties of field beans and the results compared with the reproduction on Prolific Longpod broad beans.

The aphids used in the experiments were the offspring from one Fundatrix.

The experiments were all carried out under similar conditions. Five plants of each variety were tested, and the total number of aphids produced on each plant, from one a. v. ♀, in 14 days was counted.

The mean values of infestation for the varieties range from 37 to 1037, *vide* Tables I and II.

These mean values allow of the varieties being grouped into classes representing various grades of susceptibility, ranging from 98 per cent. to 3 per cent. *vide* Table III.

Vicia narbonensis has a very low susceptibility. The results obtained indicate that resistance or susceptibility may be largely determined by genetic factors in the plant.

REFERENCES.

(1) Davidson, J. (1921). *Ann. Appl. Biol.* viii, 51–65.

(2) De Candolle, A. (1884). *Origin of Cultivated Plants*, 316–321.

APPENDIX

STATISTICAL CONSIDERATIONS INVOLVED IN TABLES I AND II OF THE ABOVE PAPER.

By R. A. FISHER, M.A.

Fellow of Gonville and Caius College; Statistician, Rothamsted Experimental Station, Harpenden.

The discussion of the probable error to be attached to Dr Davidson's aphis infestation numbers, involves points of statistical interest, which have hitherto not, in print at least, received adequate treatment.

Since only five infections were made on each of the varieties tested it is clear that only the roughest estimate of the standard deviation can be based upon the data for a single variety. For a standard deviation estimated from a sample of five is not only subject to very large errors of sampling, but is distributed in a markedly skew manner; on the other hand, the causes of variation, whatever they may be, must be closely analogous in all the varieties tested; and this fact should enable us to make use of the information supplied by the whole of the material, to estimate with some accuracy the probable error to be ascribed to each value.

The process of obtaining a single probable error from the deviations of a number of distinct groups has been applied successfully in cases where it may be assumed that the groups are equally variable; as is the case when a correlation ratio is determined on the assumption of the equal variability of the arrays. In the case of the infestation numbers no such assumption is *a priori* plausible, for setting aside the lowest mean which is evidently exceptional, the 18 means range from 286 to 1037. Moreover, an inspection of the figures for the individual plants shows that the higher numbers are, as is to be expected, actually the more variable. There exists, however, a class of distribution, of which the Poisson Series is the classical example, in which the variance is proportional to the mean.

To test whether this is the case with the infestation numbers, the quantities

$$\frac{n\mu_2}{\bar{x}}$$

were calculated for each variety, where $\bar{x}$ is the mean and μ_2 the second moment of each sample; $n = 5$ in every case but two, for which only four counts were available. In these cases the quantity was increased by one-third, and used with the others to obtain the aggregate. Adding the 18 quantities obtained from the several varieties, and dividing by 18 $(n - 1)$, we find that on the average the variance is nearly 28·5 times the mean. If now the variance is proportional to the mean we have for each variety

$$\sigma^2 = 28{\cdot}5\bar{x}$$

and the probable error is thence calculated with 18 times the weight with which it would be calculated from a single variety.

A precise test of the accuracy of the above assumption is afforded by the distribution of

$$\frac{n\mu_2}{\sigma^2}$$

for, if the standard deviations have been correctly calculated, this must be distributed as in χ^2 when $n' = 5$ in Elderton's table of Goodness of Fit. For the 17 values we have the following comparison.

	Expected $\frac{n n_2}{\bar{x}}$ m	Observed	e	$\frac{e^2}{m}$
0–2	4·49	4	− ·49	·05
2–4	5·61	8	+2·39	1·02
4–6	3·52	1	−2·35	1·03
6–8	1·83	2		
8–10	·87	1	+ ·44	·12
10–	·69	1		
				$\chi^2 = 2·22$

Since we have introduced one empirical value we must take $n' = 3$, and obtain $P = ·336$. Thus in 33 cases out of 100 we should expect a worse fit to occur by chance, and this shows that the totality of the observations shows no significant deviation from the set of standard deviations which we have assigned to them.

With a knowledge of the probable errors of the infestation numbers it is possible to test to what extent the several varieties may be grouped together as possibly identical in respect of aphid infestation. From the genetic standpoint it is of the highest importance to determine the continuity or discontinuity of susceptibility: and it is only too frequently that statisticians infer the continuity of a variable quantity without testing to what extent the apparent continuity is due to genuine continuity, due to a multitude of underlying genetic factors, and to what extent a real discontinuity has been obscured by chance variation. In the present case the homogeneity of certain groups is suggested by the mean values. Without being able to test this delicate point with rigour, it is worth while to note that the numbers obtained from certain groups are consistent with the supposition of identical susceptibility.

The group of three varieties (Nos. I, II, XIX) giving mean infestation numbers 1007 to 1037, may be regarded as samples from a single group with mean infestation number at 1020. The standard deviation of the individual plants from this value is 167, and that calculated as explained above is 171.

The variety No. III with infestation number 737 appears to be isolated.

The four varieties (Nos. IV, V, VI, VII) with infestation values 513 to 616 are distributed consistently with the supposition that they are

samples of a group with mean value at 570, the observed standard deviation is 105 while the calculated one is 127.

The nine varieties (Nos. VIII, IX, X, XI, XII, XIII, XIV, XV, XVI) with infestation numbers 370 to 451 are distributed about a mean of 407, the observed standard deviation is 103 as against a calculated value 108. The two remaining varieties (Nos. XVII and XVIII) do not seem to be assignable to any other group, and may be regarded at least provisionally as each representing a separate grade of immunity; the very low susceptibility and the high variability of the last variety (No. XVIII) especially makes it worth a more extensive study.

(*Received Oct. 25th*, 1921.)

THE TOXIC ACTION OF TRACES OF COAL GAS UPON PLANTS

By J. H. PRIESTLEY.

The fact that under certain conditions small quantities of unburnt coal gas may produce very deleterious effects upon vegetation is obviously of considerable economic importance. Both in Germany and in the United States these effects have frequently been under investigation but in this country they have attracted less attention. The earlier recognition of this type of poisoning of vegetation in other countries may be due in part to the general differences in composition between the illuminating gases employed, but the composition of coal gas in this country varies within wide limits and a change in the general methods of coal carbonisation might at any time so alter the average composition of British coal gas that this phenomenon might become of greater importance in British horticulture. Gas poisoning of vegetation probably occurs at present in this country but, as the American workers have found (5, *loc. cit.* p. 28), the absence of specific diagnostic characters enabling the damage to be accurately assessed, makes it difficult to appraise the economic importance of this particular source of injury to plants.

As the result of experiments in another field of investigation, the writer's attention was drawn to some of the cases of injury produced experimentally upon plants by the use of coal gas, and the structural changes resulting from gas poisoning were therefore examined. The preliminary results of this examination appear so significant that they are presented in this paper.

As so little attention has been devoted to the subject in this country, a brief summary is first given of the recorded effects of gas poisoning and of the definite information obtained by both German and American workers as to the constituents in the gas responsible for the toxic action. Very varied phenomena have been discussed in connection with the subject and throughout this paper attention will be restricted to cases where injury is reported as the result of the action of relatively low concentrations of coal gas. In such cases a common cause for the toxic effect produced seems to be clearly demonstrated. On the other hand

when coal gas in high concentration displaces the air normally present around and within the plant, secondary effects may well be produced, such as asphyxiation, as found by Kosaroff(7) in his experiments with hydrogen and carbon dioxide.

The Nature of the Injuries produced by Traces of Coal Gas.

In the normal flowering plant investigators are agreed that the organ most sensitive to the toxic action of traces of the gas, is the root(5, 1, 3). The normal leafy stem appears to be very little affected. Knight and Crocker(6) have drawn attention to the extraordinary sensitiveness of etiolated pea epicotyls, whilst Harvey(4) has shown that the growing leaves of Ricinus are exceedingly sensitive and Crocker and Knight(2) report similar behaviour with the flower buds of carnation. The reactions of these sensitive plant structures occur at astonishingly low concentrations of the gas and thus support the earlier observations of German workers on the deleterious effects produced by laboratory air contaminated with small quantities of unburnt gas, upon the growth of etiolated seedlings of the pea, vetch, or lentil(9, 15) and upon the etiolated shoots of the potato(16).

As the result of a great deal of work, mainly by the investigators quoted above, there is general agreement both as to the structural effects produced upon the plant and as to the constituents of the gas responsible for the effect. There is also agreement that the actively growing organs of the plant are far more sensitive than resting structures.

In the case of roots, tubercles near the tip of the growing root are reported by Doubt(3) and Harvey and Rose(5), a lessened growth in length together with local increase in girth is reported by Molisch(8), Richards and McDougal(14), whilst both Molisch(8) and Harvey and Rose(5) report irregular curvatures in the root. Similarly in the case of the etiolated stem, Neljubow(9) reported and Knight and Crocker(6) confirm, a series of reactions on the part of the plants which develop progressively with rising concentrations of the gas. These reactions in order are (1) reduction in rate of growth, seedling remaining erect, (2) diageotropic curvature with slight swelling of stem, (3) an increased swelling with further reduction of growth, (4) complete cessation of growth in length with much swollen stem curved diageotropically, (5) death of seedling. Richter(15) has studied the structure of the swollen zone of the stem and notes an abundant development of collenchyma together with numerous cracks in the surface which are lined with cork.

In the carnation the flower bud fails to open, the petals are discoloured and withered, leaving the stigmas protruding. In the leaves of Ricinus and in other leafy shoots, epinastic movements are the first indications of injury. Harvey's experiments upon Ricinus show that in this plant leaf-fall occurs with relatively very low concentrations of the gas around the leafy shoot. In other experiments where leaf discoloration or leaf-fall has been reported it is in all probability a secondary effect following upon damage done to the root system by the gas.

Many statements draw attention to the effect of the gas upon superficial corky tissue; Stone (17) reported proliferation of tissue at the lenticels of willow slips growing in water charged with the gas, Doubt (3) reports the development of soft spongy tissue in the lenticels of Hibiscus and Sambucus, and upon the leaf scars of Lycopersicum, also the appearance of deep longitudinal cracks in the bark of many woody plants, apple, pear, ash, etc. Harvey and Rose (5) also noticed proliferation at the lenticels of roots when gas was slowly passed through the soil, whilst Richter (15) had noted the same effect produced by tobacco smoke.

Toxic Constituents of the Gas.

Nelbujow (9) was the first to show experimentally that the effect upon etiolated seedlings might be traced to unsaturated hydrocarbons which could be removed by passing the gas over red hot copper oxide. He also showed that ethylene in particular, if present alone at a very high dilution indeed, was capable of producing similar effects upon plants. The exhaustive experiments of Crocker and his colleagues (2, 3, 4, 5, 6) have placed this result beyond all doubt, and they show also that other possible poisonous constituents such as prussic acid, carbon monoxide, sulphur dioxide, etc. if present in the atmosphere in concentrations equivalent to that provided by the toxic amount of illuminating gas or tobacco smoke, are completely without action upon the plant. Wehmer (18, 19, 20), who has recently attributed the toxic action of coal gas, first of all to benzol and its homologues and sulphur compounds, and then later (21, 22) to hydrocyanic acid gas, appears to have been unaware of the earlier work of Crocker and his colleagues. In any case his experiments are not strictly comparable as he works with very high concentration of coal gas, in many experiments 100 per cent.; furthermore, he does not obtain toxic effects when these constituents are introduced into air at concentrations equivalent to those in which they occur in toxic concentrations of coal gas. The proportion of unsaturated hydrocarbons present in the illuminating gas

in Germany is frequently very high, whilst in America (where water gas is the illuminant) it is also usually higher than in this country; this probably provides the reason for the phenomena being first on record in Germany and then found of practical importance in America, whilst they are nearly ignored in this country.

When ethylene is used alone in air the experiments of Knight and Crocker(6) show that a concentration of one part in ten million will retard the growth of the etiolated epicotyl of the pea, whilst a concentration of four parts in ten million will produce the full triple response, retardation of growth, increase of girth and the diageotropic position. Even with the illuminating gas at Leeds, which contains a relatively small concentration of ethylene (about 2 per cent.), it is very easy to obtain the full effect when growing etiolated seedlings in a laboratory where gas is frequently used, and experimentally it can be induced with the utmost ease. It was the consideration of the experiments of Knight and Crocker upon the etiolated pea seedling that led the writer to the experimental work which may go far to explain the mechanism of the effect produced.

The Mechanism of the Toxic Action of the Gas.

The significance of the structural effects produced by ethylene upon the etiolated epicotyl of the pea was immediately evident to the writer in the light of some observations recently made on etiolated plants. This work is now being published(11), but it is necessary to state here some results obtained as to the structure of the etiolated epicotyl of the pea and as to the function of an endodermis. In conjunction with Dr J. Ewing (11 *f*) the writer has found that in many plants grown under etiolation conditions, the stem contains a well marked functional primary endodermis from the base of the stem to just behind the growing apex. In the stem of the same plant grown in the light such an endodermis is only present for a very short distance above the ground level. It will be shown elsewhere that in these plants the special structural and morphological features characteristic of etiolation, are largely the result of the presence of this endodermis.

By a primary endodermis is meant an unbroken cylinder of cells in which the Casparian strip forms a continuous network in the substance of both transverse and longitudinal radial walls. An examination of the literature shows that the presence of this primary endodermis in stems grown in the dark has occasionally been noted, but its widespread occurrence under these conditions and the significance of its presence have been

completely neglected. This may be partly due to the fact that the presence of the strip may easily be missed unless the sections are treated with a special view to its demonstration. Many methods will be found excellent for this purpose; sections cleared by boiling in potash or in Eau de Javelle, washed, and then stained in phloroglucin and hydrochloric acid, or in gentian violet or safranin, will show the strip admirably. In sections that have not been cleared, the staining reaction of the strip is more difficult to observe, but its presence is often indicated by the occurrence in the endodermal cells of contracted protoplasm which runs always tangentially across the cells remaining in contact with the walls in the region of the strip. In fresh sections stained in phloroglucin and then mounted in concentrated hydrochloric acid, the endodermis is usually very conspicuous, this arrangement of the contracted protoplasm giving it the appearance of a continuous dense thread forming a complete ring.

The writer has recently spent a considerable amount of time investigating the behaviour of the primary endodermis (Priestley (12) and (11 *c*)). Such a cylinder of tissue may be visualised as a chimney in which the bricks are protoplasts while the Casparian strip represents the mortar between the bricks. This cylinder encloses the vascular strands, within which sap is moving through the plant. Organic solutes are frequently present in the sap, which are of the utmost significance in relation to the growth of the tissue of the plant, continued merismatic growth being impossible unless these solutes are freely supplied. The sap contained within the vascular cylinder will diffuse outwards by way of the walls between the protoplasts and will thus reach the endodermal cylinder. Whether it can reach the tissues outside this depends upon the structure of the endodermis. When an endodermis has the primary structure described above the organic solutes are unable to leak out, because the endodermal protoplasts are relatively impermeable and permit very few solutes, and those mainly inorganic, to pass; whilst the Casparian strip appears to be impregnated with fatty substances and is therefore relatively impermeable both to water and to substances dissolved in water. In a stem with such an endodermis then the growth and structural development of the tissues outside the endodermis is severely restricted.

The peculiar appearance of etiolated plants with their elongated thin stems and undeveloped leaves may therefore be attributed in part to the presence of a functional endodermis within them, restricting the supply of sap with nourishing solutes to the tissues within the endodermis. This idea receives remarkable confirmation from a study of the

effects produced by illuminating gas or ethylene upon the etiolated epicotyl. The structural effects described immediately suggested that the primary endodermis was no longer being developed and that consequently further growth was accompanied by lateral leakage of the sap into the cortex through the newly differentiated stem tissue near the growing point. The result of the arrival of these solutes in the cortical region of the stem was immediately shown by increase in girth with less development in length, and, as is shown later, the diagetropic curvature might also be anticipated.

The fact that this toxic action of illuminating gas depended upon the unsaturated hydrocarbons present in it was of immediate significance in other investigations in progress. In conjunction with Miss E. North (11 *c*) the writer had arrived at the conclusion that the peculiar properties of the Casparian strip could only be explained on the assumption that it was impregnated with fatty substances. Its staining reactions showed that it was usually also lignified and that it retained acid properties. The difficulty with which the fatty substances were removed, suggested that they were condensation or oxidation products of fats or rather, in view of their acid reaction, of fatty acids. Observations with Mr R. M. Woodman (as yet unpublished) upon the broad bean had shown that the germinating seed contains at the root tip before germination 4 per cent. of fat, and that the fat included both oleic and linoleic acid, though not in sufficient concentration to cause a rapid "drying" of the extracted fat on exposure to air. The peculiar properties of the Casparian strip suggested therefore that the unsaturated linoleic acid present in the root tip, during the tissue differentiation in the region behind the growing tip, diffused from the vascular strand across the cylinder of cells representing the potential endodermis. Here in some way the unsaturated acid was picked out from the saturated acids, and thus concentrated in the walls of a layer of cells abutting upon intercellular spaces containing air, it oxidised and condensed, practically to a varnish, which impregnated the Casparian strip and was responsible for its characteristic behaviour in restricting the diffusion of an aqueous sap. Its sharp localisation at the strip suggested that the diffusing unsaturated acid must be picked up in this region by chemical means, and the acid reaction of the strip indicated that the chemical union was not with the carboxyl group. The significant observations of Neljubow, and of Crocker and Knight, suggest that in the presence of unsaturated hydrocarbons this Casparian strip may fail to form, and thus the structural change in the epicotyl may be produced. The significance of these facts is unavoidable. The

unsaturated hydrocarbons diffusing into the root, appear to saturate the chemical linkages which usually pick up the unsaturated fatty acids, so that the latter are no longer held up in the region of the future endodermis.

This argument has been placed first because it preceded in the case of the writer any experimental work with illuminating gas. It only remains to add that experiment and observation support the conclusion thus drawn. Observations were first made upon broad bean seedlings growing in darkness. The epicotyl then develops tall and turgid, round or oval in outline, with a functional primary endodermis reaching to within a centimetre of the growing point. Seedlings were grown over water in bell jars into which a few cubic centimetres of illuminating gas were bubbled. Within three days the epicotyl was evidently swelling in girth as compared with the control. In another day or two the outline of the stem in the "gassed" plant was nearly square in cross section, just as in the normal bean plant grown in daylight. This square stem, the writer had already learnt (11 *f*), to associate with the disappearance of the endodermis. With the help of Miss L. M. Woffenden a complete structural examination of the seedling was made, when it was immediately clear that the base of the swollen region coincided with the appearance of gaps in the functional endodermis, which lower down in the stem formed an unbroken ring, whilst a little further up in the swollen region every vestige of Casparian strip had disappeared. The disappearance of the Casparian strip under gas poisoning has also been seen in the etiolated epicotyl of the pea.

It was interesting to note that the gaps in the endodermis appeared first opposite the spaces between the vascular bundles. This was to be expected as the fatty acids used in the formation of the Casparian strip diffuse outwards from the vascular strands towards the endodermal region. Clearly then these acids will first be anticipated in arrival by the ethylene at points in the endodermal ring which are most distant from the vascular strands.

The observations of Richter (15) upon the histology of the swollen zone of the pea epicotyl are necessary corollaries of the disappearance of the functional endodermis. With the insurgence of the sap from the vascular strands into the cortical regions collenchyma formation follows, whilst it will be shown elsewhere (11 *e*) that the formation of active cork meristems in cortical regions requires the free access to the meristem of the organic solutes from the vascular strands.

The curvature of the etiolated epicotyl or shoot described by

Neljubow (9, 10) and Singer (16) as a diageotropic response and by Molisch (8) and Richter (15) as the combined result of decreased geotropic, and increased heliotropic sensibility, require further examination. The etiolated shoot has always a sharply curved apex which only becomes erect upon exposure to light. The sharp curvature must be an indication of different rates of growth in the two sides of the apical meristem, the inner side of the hook at the apex presumably finding greater difficulty in development. It is only natural, therefore, that differentiation behind the growing apex should also proceed at unequal rates on different sides and that as a consequence the upper rim of the endodermal cylinder should not be at the same level all round the stem. Usually it may be expected to lag behind in development on the side which develops from the inside of the hook. Naturally the failure of the endodermis in the gassed plants usually occurs at a lower level on this side, and extension of cortical tissues follows therefore most rapidly on this side, and the epicotyl is bent over with the hooked apex on the upper side of the horizontal portion. While this is the usual curvature of the horizontal stem it is by no means invariable, the position of the first break in the endodermis is also very irregular and probably determined by a varying combination of internal and external factors.

The explanation just presented of the mechanism of gas poisoning accounts adequately for the sensitiveness of the etiolated epicotyl and the insensitiveness of the normal stem in the same plant, as in the normal stem no functional endodermis is present. It also accounts adequately for the poisonous effect of traces of the gas upon roots of the higher plants where a primary endodermis is invariably present in the growing region. Here again, examination in the case of the broad bean has shown the primary endodermis broken through as a result of the effect of the gas, and the consequent tubercular swelling is clearly due to the abnormal supply of nutrient sap to the cortical tissues just behind the growing point. The importance of the primary endodermis in the development of sap pressures by the root has been emphasised elsewhere (12), and reference to these papers will show that in the opinion of the writer this action of illuminating gas would ultimately prove fatal to the plant, as the supply of sap to the growing aerial portion of the plant depends very largely upon the exudation pressures in the root.

At present the writer is not in a position to discuss the interesting effects of traces of coal gas upon the flower bud of carnation, or the epinastic movement of the leaf of Ricinus, but the effect produced upon cork (p. 148) appears to be closely related to the effect produced

upon endodermal development. The mechanism for the normal production of the Casparian strip appears to require two factors: (1) a cell membrane in a specially receptive chemical state; (2) diffusing unsaturated fatty acids. The cell membranes appear to be found in the necessary state only when recently differentiated, and in the presence of oxygen. When cork tissue is forming, the cells are laid down by a meristem, the walls of which can be shown to have many properties in common with those of the meristem at a growing point. As the cells are cut off to the outside they are in contact with oxygen, and at the same time fatty acids are deposited in them and undergo transformation into suberin (Priestley (13), Priestley and Woffenden (11 *e*)). If these acids are unsaturated, then their deposit in these membranes may well be hindered in the presence of gaseous unsaturated hydrocarbons. Under these conditions, the walls would remain unimpregnated with fatty acids, and would be readily distended, giving rise to the proliferated tissue so frequently reported in cases of gas poisoning. The tissue formed under these conditions would be extremely fragile, and as it formed at the base of the normal bark, the strain, engendered by the expanding tissue within, would cause shearing in these weak layers resulting in the development of cracks in the bark. An experimental investigation into the truth of these suggestions is now in progress.

Summary.

1. There is clear evidence in the literature that the toxic action of traces of illuminating gas upon plants may be traced to the presence of gaseous unsaturated hydrocarbons. A concentration of one part of ethylene in ten million of air is toxic to the etiolated epicotyl of a pea.

2. The effect of these unsaturated hydrocarbons can be traced in the case of root or etiolated stem to their inhibition of the formation of a functional primary endodermis, which is usually present in these plant structures.

3. The unsaturated hydrocarbons prevent the formation of a functional endodermis, by preventing the normal accumulations of unsaturated acids in the region of the future Casparian strip.

4. It is suggested that the effect of traces of these gaseous unsaturated hydrocarbons upon cork formation may be due to the arrest of the normal deposit of fatty acids in the membranes of the cork cells.

5. The practical significance of this work lies in the fact that definite diagnostic features may now be sought for when injuries to plants are suspected to be due to gas poisoning.

REFERENCES.

(1) BÖHM (1874). Ueber die Einwirkung des Leuchtgases auf die Pflanzen. *Bot. Zeit.* XXXII, 74–75.
(2) CROCKER, W. and KNIGHT, L. I. (1908). Effect of Illuminating Gas on Carnations. *Bot. Gaz.* XLVI, 259–276.
(3) DOUBT, SARAH L. (1917). Response of Plants to Illuminating Gas. *Bot. Gaz.* LXIII, 209–224.
(4) HARVEY, E. M. (1913). Castor Bean Plant and Laboratory Air. *Bot. Gaz.* LVI, 439–442.
(5) HARVEY, E. M. and ROSE, R. CATLIN (1915). The Effects of Illuminating Gas on Root Systems. *Bot. Gaz.* LX, 27–44.
(6) KNIGHT, L. I. and CROCKER, W. (1913). Toxicity of Smoke. *Bot. Gaz.* LV, 337–371.
(7) KOSAROFF, P. (1897). Einfluss ausserlich Faktoren auf den Wasseraufnahme. *Inaug. Diss.* Leipzig. (Quoted from Harvey and Rose (5).)
(8) MOLISCH, H. (1884). Ueber die Ablenkung der Wurzeln von ihrer normalen Wachstumsrichtung durch Gaze (Aerotropismus). *Sitzungsber. Kaiserl. Akad. Wiss. Wien*, XC, 111–196.
(9) NELJUBOW, D. (1911). Geotropismus in der Laboratoriumsluft. *Ber. der deutsch. Bot. Ges.* XXIX, 97–112.
(10) —— (1901). Ueber die horizontale Nutation der Stengel von Pisum sativum und einiger anderen Pflanzen. *Beih. zum Bot. Centr.* X, 128–138.
(11) PRIESTLEY, J. H. (1922). Physiological Studies in Plant Anatomy appearing in *New Phytologist*, vol. XXI, as follows:
(11 *a*) —— . Introduction.
(11 *b*) —— and ARMSTEAD, DOROTHY. The Physiological Relation between the Xylem and the surrounding Tissues.
(11 *c*) —— and NORTH, EMILY E. The Structure of the Endodermis in relation to its function.
(11 *d*) —— and TUPPER CAREY, ROSE M. The Water Relations of the Plant Growing Point.
(11 *e*) —— and WOFFENDEN, LETTICE M. Causal Factors in Cork Formation.
(11 *f*) —— and EWING, J. Etiolation.
(12) —— (1920). The Mechanism of Root Pressure. *New Phytologist*, XIX, 191–200, and 1922, XXI, 41–47.
(13) —— (1921). Suberin and Cutin. *New Phytologist*, XX, 17–29.
(14) RICHARDS, H. H. and MACDOUGAL, D. T. (1904). The Influence of Carbon Monoxide and other gases upon plants. *Bull. Torr. Bot. Club*, XXXI, 57–106.
(15) RICHTER, OSWALD (1903). Pflanzenwachstum und Laboratoriumsluft. *Ber. der deutsch. Bot. Ges.* XXI, 180–194.
(16) SINGER, MAXIMILIAN (1903). Ueber den Einfluss der Laboratoriumsluft auf das Wachstum der Kartoffelsprosse. *Ber. der deutsch. Bot. Ges.* XXI, 175–180.
(17) STONE, G. E. (1913). Effect of Illuminating Gas on Vegetation. Report of the Botanist. 25*th Annual Report Mass Agric. Expt. Station*, pp. 13–28.
(18) WEHMER (1917). Leuchtgaswirkung auf Pflanzen. I. Die Wirkung des Gases auf Sporen und Samenkeimung. *Ber. der deutsch. Bot. Ges.* XXXV, 135–154.
(19) —— (1917). II. Wirkung des Gases auf grüne Pflanzen. *Ber. der deutsch. Bot. Ges.* XXXV, 318–332.
(20) —— III. Wirkung des Gases auf Wurzeln und beblatterte Zweige beim Durchgang durch Erde oder Wasser. *Ber. der deutsch. Bot. Ges.* XXXV, 403–410.
(21) —— (1918). IV. Die Wirkung des Gases auf das Wurzelsystem von Holzpflanzen; Ursachen der Gaswirkung. *Ber. der deutsch. Bot. Ges.* XXXVI, 140–150.
(22) —— (1918). V. Wirkung auf Holzpflanzen; Blausaure als schädlichster Gasbestandteil. *Ber. der deutsch. Bot. Ges.* XXXVI, 460–464.

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COMMON SCAB OF POTATOES[1]

PART I

By W. A. MILLARD, B.Sc.

(*Lecturer in Agricultural Botany and Adviser in Mycology, University of Leeds.*)

(With Plates VIII, IX.)

Common Scab has long been one of the most widespread of potato diseases and is recorded as early as 1825. In spite of this, it has hitherto received little attention from mycologists in this country. This neglect may be attributed possibly to the fact that, although the disease occurred in all parts of the country, it was only in certain localities that it appeared with sufficient virulence to make it a serious problem. In such places, as for example, in certain districts in the great potato-growing county of Yorkshire, the crop is sometimes rendered so unsightly by the Scab that it is practically unsaleable. In consequence, many farmers have been obliged to omit potatoes from their rotation on land which, from a yield point of view, was eminently suitable for them.

The distribution and virulence of the disease appear to be closely related to the type of soil on which the crop is grown, occurring chiefly on soil of a light sandy or gravelly nature, to a much lesser degree on heavy soil, and rarely, if at all, on true peaty soils.

Accounts of Common Scab are numerous in American literature, but, in many respects, these appear to be incomplete when applied to the disease in this country and a short description will therefore be given here.

Description of Common Scab.

The disease first appears in the form of small brown spots on the skin of the tuber. These increase in size and, at the same time, the tissue of the potato immediately below them becomes brown and pulpy. At this early stage, the dark brown surface of the spot remains smooth and unbroken and on it a delicate greyish white mycelium is produced. This rapidly disappears when the potato is lifted and exposed to light.

[1] A grant in aid of publication has been received for this communication.

The scabs increase to an indefinite size, two or more often coalescing, but, generally, before a diameter of about 4 mm. has been reached in any individual scab, its covering skin ruptures and a shallow depression is exposed. Very quickly, however, the base and edges of the scab become thickened with layers of cork laid down by the potato in its attempt to cut off the disease from the underlying tissue.

The mature scabs vary considerably in general appearance. In some, the shallow depression formed in the early stages of the disease is never afterwards raised to the surface by the subsequent formation of cork and the affected potato presents a pitted appearance. In others, the scabs are raised by the abundant cork formation and stand out above the surface of the tuber in knob-like projections. These two forms of Scab, which we may call "pitted" and "raised" respectively, appear to be the outstanding types of the disease when it occurs in its most virulent form.

The commonest form of Scab in this country, however, is intermediate between these two extremes (Fig. 1). It is slightly raised and is also characterised by an irregularly concentric series of wrinkled layers of cork arranged around a central core or depression.

These different types, together with others showing greater or lesser variations, will be considered more fully in a later paper.

Cause of Scab. The earliest reference to Common Scab is found in Loudon's *Encyclopaedia of Agriculture* (1825) and reads as follows: "Scab, that is to say, the ulceration of the surface of the tubers, has never been explained in a satisfactory manner. Some attribute it to the Ammonia from the dung of the horse, others to alkali, and certain others to the use of wood ashes in the soil."

In 1884, the idea was introduced by W. G. Smith(1) that Scab was produced by the irritating action of sharp or gritty particles in the soil on the swelling tubers. Much support was lent to this theory by the fact that Scab was certainly most prevalent on soils of a light gritty nature and by the generally accepted belief that ashes produced Scab.

In 1890, however, an organism was isolated in America from a form of Scab known as "Deep Scab" by Thaxter(2) who proved it to be the causative organism of the disease, and gave it the name "Oospora scabies." The nomenclature of the fungus and its place in the systematic scale have since passed through many changes. In 1912, Cunningham(3) placed it in the genus Streptothrix. In 1914, Güssow(4) transferred it to the genus Actinomyces, and Lutman and Cunningham(5) believed it to be identical with *Actinomyces chromogenus* (Gasperini).

Later, however, the work of Krainsky(6), Conn(7) and particularly of Waksman and Curtis(8) showed that the characters of *A. chromogenus* were those of a group rather than of a species. Finally, in 1919, Drechsler(9) made an exhaustive study of the morphological character of the genus Actinomyces and concluded that it should be placed in the Hyphomycetes as a Mucedinous group. He therefore re-named Thaxter's organism *Actinomyces scabies* (Thaxter—Güssow).

Thaxter's original work was only slowly recognised in this country and this was possibly due in part to our ingrained belief in the theory of Mechanical Scab. We may attribute it, however, with more credit to ourselves, to the fact that from Thaxter's own account and the photographs which accompanied it, it was by no means certain that the disease, which he called "Deep Scab" was the same as the "Common Scab" familiar in this country. So much indeed was this the case that Massee(10) in 1910, describes the American Scab and says: "I have but rarely observed this disease in this country."

The writer now realises that Thaxter's "Deep Scab" was the form of Common Scab which he has here named "pitted" and as already has been pointed out, this type of Scab is not the most common in this country. Later American writers have published confirmatory accounts of Thaxter's work in which photographs of scabbed potatoes appear that might well replace our own photograph of typical Scab in Fig. 1. Unfortunately, however, no details of the inoculation experiments appear proving the pathogenicity of the organisms isolated and no photographs of the results of any such experiments. Lutman and Cunningham(11), for example, having isolated species of Actinomyces from scabbed potatoes from various sources, state that these agreed, with a few minor differences, with Thaxter's organism and add "all were found capable of producing scab on inoculation." It seems very surprising that in such an early stage of our knowledge of Scab no details or photographs of these successful inoculations should have been given.

Again, McKinney(12) says: "The writer has studied three strains of the scab organism isolated from scabby potatoes grown in three localities in this state, *all of which* are pathogenic upon growing tubers." No data of the experimental proof of this statement are given, however, although, in this case, the omission is excusable on the grounds that the paper deals only with the nomenclature of the Potato Scab organism.

There appears indeed to be no complete confirmation of Thaxter's work, which he himself called "preliminary" in either American or English literature.

On the other hand, it may be recalled that in 1890, Bolley(13) isolated a bacterium which he claimed was able to reproduce Scab and pointed out that in choosing his material for isolation of the organism, he avoided such scabs as were "blackened or pitted."

Again, in 1915, Pethybridge(14) writing on the "Supposed causes of Brown Scab," refers to Thaxter's organism and says: "Although some writers have gone so far as definitely to ascribe some of our scab to this organism, no scientific evidence appears yet to have been published proving that the said organism is really responsible for our ordinary potato scab."

In 1907, a contribution to our knowledge of the subject was made by Seton and Stewart(15). These investigators showed that Common Scab could not be produced in sterilised soil and this work was followed in 1915 by Pethybridge and Fannin(16) who proved conclusively that the theory of mechanically produced Scab was untenable.

This work received confirmation from some unpublished experiments carried out in 1914 by the present writer, and in 1915 by Mr T. Laycock, then Assistant Lecturer in Agricultural Botany at this University.

In view therefore of the general uncertainty on the subject in this country, it was thought highly desirable to make an *a priori* investigation of the cause of Common Scab and this was carried out concurrently with some work on remedial measures, a popular report(17) of which has already been published.

Isolation of the Organism of Common Scab.

This was begun in 1916, and in the subsequent absence of the writer on war service, the primary work of isolation together with the study of the morphological and cultural characteristics of the original cultures obtained was carried out by Miss K. Sampson, then Assistant Lecturer in Agricultural Botany at this University. The writer takes this opportunity of expressing his admiration of Miss Sampson's excellent work in this respect and his gratitude to her for placing her notes and cultures at his disposal on his return.

In the selection of material no choice was made of any particular type of scab, but scabbed potatoes were taken from various samples which came in in the ordinary course of advisory work. No attempt was therefore made in this early work to establish any relation between the type of scab and the culture strains isolated.

The material for inoculation was taken in the majority of cases from young unruptured scabs, but a few mature and ruptured scabs were also

used as sources of inoculum. The potatoes were thoroughly scrubbed under the tap, and allowed to dry. The inoculum was then taken from the closed scabs by lifting the covering skin with a sterilised scalpel and removing a small amount of the soft tissue below with a platinum loop. In the case of the open scabs the corky edge was cut away and a scraping taken from the exposed tissue.

The medium used was Potato Agar and the results of the plating are best described in Miss Sampson's own notes on a few of the cases.

Variety of potato	Description of scab	Notes on the plates poured
British Queen	Unruptured	Plate crowded with lichenoid colonies forming a greyish mass over the whole surface and staining the medium dark brown
May Queen	,,	Plate crowded with light grey lichenoid colonies producing a dark brown stain on medium. 12 or more bacterial colonies and 1 mould also present
May Queen	,,	Plate thickly seeded with light brown or grey lichenoid colonies. Greyish brown stain spreading through medium
Great Scot	Ruptured; open	Numerous lichenoid colonies on plate resembling those on British Queen plate

These notes, in themselves, afford strong presumptive evidence that the lichenoid colonies forming so dominant a feature of the plates were those of the causative Scab organism. In appearance, the colonies are glistening and refractive and generally so hard that it is necessary to cut them out from the medium with the platinum loop if transfers are to be made at an early stage of growth. Later, the colonies may become coated with an aerial mycelium (usually greyish white to white on potato agar) from which sub-cultures are easily made. They may be distinguished with certainty from bacterial colonies by examining the plate under the low power of the microscope, when the thread-like and often spiral extremities of their filaments are easily seen.

In all, 10 pure cultures were obtained from the different varieties of scabbed potatoes shown below.

No. of culture	Variety of potato	
1	Dalhousie	
2	British Queen	Isolated from the same scab but showing differences in culture
3	,,	
4	May Queen	
5	,,	
6	Great Scot	
7	,,	Isolated from different scabs on the same potato
8	,,	
9	Witch Hill	
10	,,	

With the exception of No. 1 which was isolated in 1916, all the cultures were isolated in August, 1918. Further examination of the morphological and cultural characteristics of the cultures made it certain that the organisms were members of the Actinomyces group, but, from the first, considerable variations were observed between the 10 strains.

An effort was made to compare them with the different species described by Krainsky (6), Conn (7) and Waksman and Curtis (8), but in no single case could complete agreement be found. This, however, is scarcely surprising since, at the time when this work was being carried out, the study of the Actinomycetes was so recent and the available literature on the subject so scanty that the identification of species was supremely difficult if not impossible. Moreover, it was soon discovered that the characters of any culture underwent considerable variation with age, conditions of growth and with extremely slight differences in the composition and reaction of the media on which they were grown.

Since this time valuable contributions have been made to the subject, on the morphological side by Drechsler (18), and on the cultural side by Waksman (19). The latter, in particular, has now placed the work of distinguishing species on a sound basis by using only media of definite chemical composition and degree of acidity throughout his cultures.

A number of species are thus now clearly defined, but in the case of *Actinomyces scabies* this is still by no means the case.

Thaxter's (2) excellent description was of necessity incomplete. Lutman and Cunningham (11) found what they considered "minor" differences between various strains which they isolated, and, as before stated, placed these strains in the species *A. chromogenus* (Gasperini).

Finally, Waksman (19) in 1919 described a type of *A. scabies* based on his own isolations of the organism but admits that a number of cultures received from other investigators differ very considerably from his own.

In view of this apparent variation in the species or group species, as the case may be, the writer felt no difficulty in considering the 10 strains isolated by Miss Sampson as true *A. scabies*, provided their pathogenicity to potatoes could be proved.

Inoculation Experiments.

These were carried out in unglazed pots 14 ins. in diameter, which were filled with soil to within 2 ins. of the top and sterilised in the autoclave at 130° C. for 1 hour. Under these conditions it was found that the temperature of the soil interior was raised to just over 100° C., which

according to Waksman (20) would suffice to kill all Actinomyces present with the possible exception of *A. invulnerabilis*. It might not however preclude some bacterial infection.

The potato sets were sterilised by immersion in one-sixth per cent. Formaldehyde for two hours, and at the time of planting three sterilised glass tubes of $\frac{1}{2}$ in. bore were inserted obliquely into each pot for the purpose of inoculation.

The varieties of potatoes planted and the strain of Actinomyces with which each was inoculated is shown in the following table:

No. of pot	Variety	No. of culture strain
1	Dalhousie	1
2	British Queen	2
3	,,	3
4	,,	1st control—uninoculated
5	Great Scot	6
6	,,	7
7	,,	2nd control—uninoculated
8	May Queen	4
9	Witch Hill	9
10	British Queen	1
11	,,	3rd control—unsterilised soil and tuber. Uninoculated

The first inoculation was made on July 30th when the plants were about 10 ins. high, and two further inoculations at intervals of one month.

The greatest care was taken throughout the experiment to keep the greenhouse as sterile as possible and floor and benches were washed with disinfectant each time the house was entered.

Results of Inoculation.

Before tabulating these, a distinction must be made between obvious, typical scabs and spots or points of infection which could only be ascribed to the inoculation after microscopic and cultural examination. These spots appeared to coincide in every case with a lenticel. They were brown to black in colour, of a diameter from 1 to 3 mm. and were very numerous on all the inoculated potatoes. Plate cultures made from the more conspicuous consisted of either all Actinomyces colonies or of Actinomyces and bacteria, and where these were found the spots were considered as definite infections and described as scab spots. Some of the more minute spots, however, which were such as are very commonly found in the lenticels of potato tubers gave bacterial colonies only.

No. of pot	Infection
1, 2, 6, 10	In each of these pots the tubers showed typical scabs from 3 to 6 mm. in diameter. In Pot 2 many of these were covered with the delicate greyish white mycelium so characteristic of the disease. In addition, large numbers of scab spots were present.
3, 5	No mature scabs present, but the tubers were covered with scab spots.
8, 9	These two crops were lifted 5 weeks before the others. No scabs were present. A few spots were shown by the May Queen tubers but these were not examined.
4, 7	Controls. Potatoes clean and glossy. In Pot 7 a few minute spots were present on the tubers but were found to be of bacterial origin only.
11	One or two small but typical scabs of diameter from 4 to 7 mm. were present on most of the tubers. This control pot was included in the series in order to ascertain the extent to which scabbing would occur normally in pots. The soil was known to produce scab badly in the open. It would appear, therefore, from the results that the conditions of pot culture are unfavourable to scab production and this is probably due to lack of aeration. The degree of scabbing produced in the inoculated pots cannot therefore be taken as any indication of the virulence of the Actinomyces strains under normal conditions.

Photographs of the scabbed potatoes from Pots 2 and 6 and of the clean potatoes from the control Pot 4 are given in Figs. 2, 3 and 4 respectively.

From these experiments, therefore, it will be seen that out of the seven strains of Actinomyces tested, five proved to be pathogenic and the negative results given by the remaining two may have been due to the early ripening of the potato varieties (May Queen and Witch Hill) concerned. The positive result in Pot 10, where an Actinomyces strain isolated from a Dalhousie potato was used as inoculum for British Queen potatoes shows that this strain is not specific to one kind of potato and this is probably true of the other strains also.

The experiments thus fully confirm Thaxter's original work and show its applicability to types of scab in this country. They show, in addition, that Common Scab may be produced by various members of the Actinomyces group exhibiting considerable differences in culture. To what extent these different members may be considered as belonging to a single species or species group is however a matter needing further investigation. Some work on the subject is now being carried out and it is hoped to publish the results of this in due course.

The writer wishes to express his thanks and indebtedness to Professor R. S. Seton for the kind interest he has taken in the work, to Mr S. Burr, Demonstrator in Agricultural Botany at this University, for his valuable assistance, and to Mr J. Manby, University Photographer, for the photographs.

REFERENCES.

(1) Smith, W. G. (1884). Text-book. *Diseases of Field and Garden Crops.*
(2) Thaxter, R. (1890). *Ann. Report Conn. Agric. Exp. Stn.* 80–95.
(3) Cunningham, G. C. (1912). *Phytopathology*, II, 97.
(4) Güssow, H. T. (1914). Ref. *Phytopathology*, IX, 327.
(5) Lutman and Cunningham (1914). *Vermont Agr. Exp. Stn. Bul.* 184, 42.
(6) Krainsky, A. (1914). *Centbl. Bakt.* Abt. 2, Bd. XLI, 649–688.
(7) Conn, H. J. (1917). *N. Y. State Agric. Exp. Sta. Tech. Bul.* 60.
(8) Waksman and Curtis (1916). *Soil Sci.* I, 99–134.
(9) Drechsler, C. (1919). *Bot. Gazette*, LXVII, 65–83 and 147–168.
(10) Massee, G. (1915). Text-book. *Diseases of Cultivated Plants and Trees*, 458.
(11) Lutman and Cunningham (1914). *Vermont. Agr. Exp. Sta. Bul.* 184, 24.
(12) McKinney, H. H. (1919). *Phytopathology*, IX, 328.
(13) Bolley, H. L. (1890). Ref. *Ann. Report Conn. Agric. Exp. Sta.* 81 and 84.
(14) Pethybridge, G. H. (1915). *Journ. Dept. of Agric. and Tech. Instr. for Ireland*, XV, 521.
(15) Stewart, J. G. (1907). Univ. of Leeds and Yorkshire Council for Agric. Educ. *Report* 70, 15–16.
(16) Pethybridge, G. H. (1915). *Journ. Dept. of Agric. and Tech. Instr. for Ireland*, XV, 522–4.
(17) Millard, W. A. (1920). Univ. of Leeds and Yorkshire Council for Agric. Educ. *Report*, 118.
(18) Drechsler, C. (1919). *Bot. Gazette*, LXVII, 65–83 and 147–168.
(19) Waksman, S. A. (1919). *Soil Sci.* VIII, 71–215.
(20) —— (1919). *Soil Sci.* VIII, 83.

EXPLANATION OF PLATES VIII AND IX

Fig. 1. A typical example of the commonest form of Common Scab. Reproduced from *Report* 118, Univ. of Leeds and Yorkshire Council for Agric. Educ.

Fig. 2. "British Queen" potatoes—the produce of Pot 2 showing scabs in various stages produced by inoculation with *Actinomyces scabies*, strain No. 2.

Fig. 3. "Great Scot" potatoes from the produce of Pot 6 showing scab spots produced by inoculation with *Actinomyces scabies*, strain No. 7.

Fig. 4. "British Queen" potatoes, the produce of the uninoculated Pot No. 4 (Control).

(*Received March 9th*, 1922.)

Fig. 1.

Fig. 2.

Fig. 3.

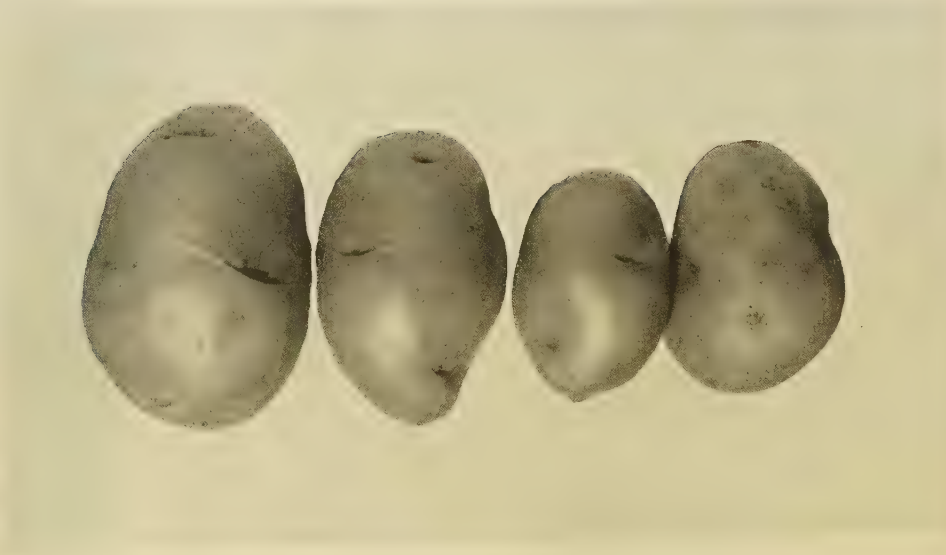

Fig. 4.

ADDITIONAL HOST PLANTS OF *OSCINELLA FRIT*, LINN. AMONG GRASSES.

By NORMAN CUNLIFFE, M.A. (Cantab.),

Christopher Welch Lecturer in Economic Zoology, University of Oxford.

The following observations supplement those recently recorded(1) relating to the utilisation of certain grasses as host plants by *O. frit*. As, in previous experiments, positive results even with the same species of grass were decidedly irregular, it was concluded that the small scale of pot experiments might be unduly influencing reproduction. These flies are difficult to rear, owing to their sensitiveness to unfavourable environmental factors. Therefore in 1920 and 1921 outdoor muslin cages measuring $6' \times 6' \times 4'$ were given a trial but, although the conditions were apparently more favourable for oviposition they were not ideal, as emergence figures were still small. In addition to determining which grasses *O. frit* could utilise as host plants, it was desired to obtain some evidence as to the relative preference for different hosts at different periods of the year.

Grasses Utilised in Winter. Experiments I to III.

Briefly the history of the host plants used in the following experiments was as follows: the seeds were sown on 23. iii. 20 under protective muslin cages, the plants being cut down to a height of 4 inches on 1. vi. 20, planted out in 9 inch pots (four roots per pot) on 29. vii. 20 and on the same day recut to a height of 4 inches to induce tillering. The pots were sunk to the brim in a shallow pit under one of the large cages, five pots of each species of grass used being distributed as evenly as possible therein. The parent flies, bred from oat grains, were divided into four lots and introduced through each of the four sides of the cage, water and food (in the form of sugar) being present in the cage in abundance. Thus, as far as possible, the effect of sluggishness on the part of the fly was eliminated, and also the separation of the host plants was facilitated when it became necessary to cage them separately for recording emergence. The small cages were examined at intervals of three or four days, and the newly emerged flies removed. Owing to the small numbers only the total emergence from each species of host plant over each interval is shown below.

Experiment I. The following grasses having given positive results in the winter of 1919 and spring of 1920 were grouped in one cage: (1) *Alopecurus myosuroides*, (2) *Festuca pratensis*, (3) *Lolium italicum*, (4) *Lolium perenne*, (5) *Poa annua*, (6) *Arrhenatherum avenaceum*. These grasses were infected on 16. viii. 20 with 148 flies, the pots being caged separately on 2. iii. 21. *Poa annua* and *Alopecurus myosuroides*, being annuals, had died out by March, 1921. The plants showed signs of attack in the autumn, but the larvae failed to subsist either in the debris or the soil after the death of the host plants.

Only 12 flies emerged in the spring of 1921—from *Lolium perenne*, 1 fly after 287 days and from *Arrhenatherum avenaceum* 1, 2, 5 and 3 flies after 267, 276, 287 and 295 days respectively, reckoning from the date of infection.

Experiment II. Holcus lanatus, *Bromus sterilis*, *Dactylus glomeratus*, *Phleum pratense*, *Hordeum murinum*, *Arrhenatherum avenaceum* var. *bulbosum* and *Avena flavescens* gave negative results in 1919–20, although it has been recorded that *O. frit* may oviposit in spring on these grasses. Having been grouped under one cage, these grasses were infected with 175 flies in 16. viii. 20 and caged separately on 2. iii. 21.

Again a very small number of flies, namely 13, emerged in the spring of 1921, but here they were spread over four host plants. *Holcus lanatus*[1] produced 1 fly after 295 days, *Bromus sterilis* 1 fly after 267 days, *Dactylus glomeratus* 1 fly after 276 days and 2 flies after 287 days, while from *A. avenaceum* var. *bulbosum* 8 flies were obtained, namely 1, 3, 3 and 1 after 267, 276, 287 and 295 days respectively.

The minimum, maximum and mean periods required for the production of the spring generation were 267, 295 and 284 days respectively, the average period being 30 days longer in 1920–21 than in 1919–20, although the dates of infection were practically the same in the two cases.

It is of interest to note that in each of these experiments the majority of the flies emerged from the *Arrhenatherum* species, approximately 90 per cent. being obtained from *A. avenaceum* in the first experiment and 70 per cent. from its variety *bulbosum* in the second.

[1] *Exp. III.* Small quantities of the following grasses, grown from seed, were caged separately in August, 1920: *Agropyron repens*, *Agropyron caninum*, *Agrostis alba*, *Aira caespitosa*, *Anthoxanthum odoratum*, *Alopecurus pratensis*, *Bromus mollis*, *Bromus erectus*, *Cynosurus cristatus*, *Festuca pratensis*, *F. sciuroides*, *F. ovina*, *Poa pratensis* and *P. trivialis*. Twenty-five flies were introduced into each cage between Aug. 18th to 31st, but in the spring of 1921 the results were negative. A spare pot of *Holcus lanatus* placed in this series produced one fly on 13. vi. 29.

Previous experiments have shown that *O. frit* may, during the winter period, breed on *Alopecurus myosuroides*, *Lolium italicum*, *L. perenne*, *Hordeum pratense* and *Arrhenatherum avenaceum*. In addition to these therefore, *Holcus lanatus*, *Bromus sterilis*, *Dactylus glomeratus* and *A. avenaceum* var. *bulbosum* may be utilised during the winter. Miles(2) states that, in the winter period, he obtained larvae of *O. frit* in *Trisetum* (*Avena*) *flavescens*, *A. avenaceum*, *Agrostis stolonifera* (a variety of *A. alba*), *Holcus lanatus* and *L. perenne* in the field, without indicating which host was the most heavily infected.

GRASSES UTILISED IN SPRING. EXPERIMENTS IV AND V.

In the spring of 1921 small quantities of the twenty-five grasses used in the first three experiments were available, as well as a few roots of the following species: *Brachypodium sylvaticum*, *Avena pratensis*, *Festuca rubra* and *Hordeum pratense*. Single pots of each of these species were placed under a large cage on 4. iv. 21, infected with 149 flies from the field on 18. v. 21 and separately caged in the middle of June.

Reckoning from the date of infection the emergence of the next generation of flies was as follows: *Agrostis alba*, 2 flies after 62 days; *A. avenaceum* var. *bulbosum*, 2 and 1 flies after 42 and 59 days; *Hordeum murinum*, 12, 7 and 1 flies after 55, 59 and 62 days respectively, while in the same periods *L. italicum* gave 1, 2 and 1 flies only; *Poa trivialis*, 4 flies after 55 days[1]. Thus, *O. frit* may exist on *Agrostis alba*, *A. avenaceum* var. *bulbosum*, *Hordeum murinum* and *Poa trivialis* during the spring period as well as in *A. avenaceum*, *Festuca pratensis*, *L. italicum*, *L. perenne* and *Poa annua*, as shown by previous experiment.

A similar experiment conducted in July gave negative results, a period of high temperature occurring during this month, causing the premature death of the parent flies.

Experiment V. In the spring of 1921 the cereals, oats, wheat, rye, barley and maize together with the grass *Lolium italicum* were grouped in the same way as the grasses in the first experiment, five pots of each species being used to ascertain whether the fly in captivity showed any decided preference for oats. The cage was infected on 18. v. 21 with 56 flies from the field and the pots separately caged in the middle of June. The emergence of flies was small, wheat, rye, barley and maize yielding none, *L. italicum* 1 and 4 flies after 50 and 59 days, oats, 1, 1, 1, 4 and 3 flies after 47, 50, 59, 67 and 73 days respectively.

[1] A pot of *Secale cereale* included in the group was comparatively heavily infected, 5, 1 and 1 flies emerging after 55, 59 and 73 days respectively.

Experiments IV and V gave minimum, maximum and mean periods of 42, 73 and 58 days (55 flies) between the times of emergence of consecutive generations in the spring, confirming the figures obtained in 1920.

The foregoing records indicate that *O. frit* in captivity does show some preference for *Arrhenatherum* species among the grasses and oats among the cereals, although owing to the small yields no comparative measure of preference has resulted.

Additional observations on the prevalence of the fly in the field, collected during the year 1921 indicate that for the years 1919 to 1921 during which the meteorological conditions were very different, the periods of high and low prevalence tend to be constant. If this conclusion is supported by another season's observations, it will probably render possible an explanation of the fact that early sown crops suffer least damage.

REFERENCES.

(1) Cunliffe, N. (1921). "Preliminary Observations on the habits of *Oscinella frit*, Linn." *Ann. App. Biol.* viii, No. 2, 105–134.

(2) Miles, H. W. (1921). "Observations on the Insects of grasses and their relation to Cultivated Crops." *Ann. App. Biol.* viii, Nos. 3 and 4, 170–181.

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STUDIES IN BACTERIOSIS. VI

BACILLUS CAROTOVORUS AS THE CAUSE OF SOFT-ROT IN CULTIVATED VIOLETS

By MARGARET S. LACEY.

(*From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.*)

A DISEASE of some considerable importance occurred in the early part of 1921 at the Hayden Violet Grounds, Stourpaine. When received, the plants were in an advanced state of decomposition; the whole interior of the stem was reduced to a soft white mush, and the rot was spreading up the petioles; several of the leaves had already fallen off owing to decay at the base of the petioles, and others were dying from the same cause.

Owing to the advanced state of decay considerable difficulty was experienced in isolating the causal organism, but eventually a pure culture of a white organism which produced vigorous rotting of vegetables was obtained.

Inoculation Experiments upon Cultivated Violets.

Underground stems, "runners," and the apices of stems were used in these experiments. In all cases, rotting of the tissues round the point of inoculation was obtained. In the case of stem infections the rotting proceeded rapidly, after 48 hours the petioles had been attacked and the leaves were dying, after a week a rotted mass from which the leaves had fallen was all that remained. In all cases the controls remained healthy. The organism also produced white rot of carrots, turnips, potatoes and onions.

Identification of the Causal Organism.

The organism was carefully compared with the laboratory culture of *Bacillus carotovorus* (strain obtained from Prof. L. R. Jones in 1911). The latter had an average length of 1·2μ and varied from 1–1·6μ, whereas the violet strain had an average length of 3μ and varied from 1–4μ with occasional larger cells up to 10μ. Apart from this difference

in size the two organisms appeared to be the same, the cultural and physiological features agreed very closely and one has no hesitation in ascribing the disease of violets to that omnivorous organism *Bacillus carotovorus*.

In the first instance the diseased plants were submitted to the Royal Horticultural Society's laboratory at Wisley, where the bacterial origin of the disease was suspected by Mr W. J. Dowson, who referred the matter for further investigation to Dr S. G. Paine to whom the author is indebted for his constant help throughout the work.

(*Received Nov. 14th*, 1921.)

REVIEW

Annals des Épiphyties. Edited by Marchal, P. and Foex, E., Tome VII, pp. 1–464 + lxxxvii. Mémoires et Rapports présentés au Comité Supérieur des Stations de Recherches en 1919 et 1920. Ministère de l'agriculture, Paris, 1921. 25 frs.

The volume is planned as in previous years, an interesting summary of the occurrence in France of plant diseases of physiological nature and due to insect, fungus and bacterial parasites occupying pages i-lxxxviii, and brief reports on the activities in the several laboratories of plant pathology and entomology in France filling the last twenty pages. Of the nineteen original papers the first (pp. 1-115) is by G. Arnaud and is a re-grouping into a new family, the Parodiellinaceae of all the Pyrenomycetes possessing internal mycelia with definite haustoria, parasitic especially on the leaves of higher plants. Most of the family have also external mycelia and contain a bright soluble pigment. The genera so grouped include Bagnisiopsis, Parodiellina, Chevaliera, Parodiopsis, Perisporina, Nematothecium, the Erysiphaceae, Exosporina, Septoidium, Ovulariopsis, Oidiopsis and Oidium. The thesis is elaborated in some detail and the memoir, which is illustrated by 25 text-figures and 10 plates, contains much matter of general mycological interest. In pp. 117–167, P. Vayssière gives an account of the campaign against the epidemic of Moroccan locust (*Dociostaurus Maroccanus*) in Crau in 1920. The procedure adopted is described in detail, perhaps the most striking item being the use of "lance-flammes," the programme for the 1921 offensive is outlined and the paper is illustrated by 11 plates. Pages 169–236 are occupied by papers by A. Paillot and by J. Feytaud on the simultaneous control of insect and fungus pests of fruit trees by mixed sprays. Arsenical-lime-sulphur, Bordeaux mixture plus arsenate of lime, Bordeaux mixture plus lead arsenate and lime-sulphur plus lead arsenate are recommended. An interesting paper is contributed (pp. 237–266) by L. Chopard on the biology and control of the Argentine ant (*Iridomyrmex humilis* var. *arrogans*) in the south of France. Pages 267–314 are occupied by a number of papers on potato diseases by H. M. Quanjer, E. Foex, J. Aumiot, E. Blanchard and C. Perret. The chief problems in question are Mosaic disease, Leaf Curl and allied troubles, and views

supporting Quanjer's hypotheses and in opposition to them are expressed by the several investigators. J. Feytaud contributes a paper describing the effect of variations of temperature, humidity, etc. on the Eudémis and the Cochylis in the Bordeaux region in 1918 and 1919. In pp. 339–370 P. Vayssière gives an interesting general account illustrated by eight plates of the insect pests of cultivated plants in Morocco, together with notes on Insecticides. Three short papers by R. Régnier describe the entomological research station at Rouen, the damage to Poplar trees caused by the leaf hopper *Idiocerus populi*; and the destructive activities of various species of Corvus in Normandy. Short accounts are given of some biological observations on the olive fly (*Dacus oleae*) and its parasites by R. Poutiers and L. Turinetti, and of the successful employment of chlorpicrin as an insecticide by P. Schindler and B. Trouvelot. There are also papers on fungus diseases of apricots by J. Chifflot and on Fusariose and two other diseases of the melon by J. Dufrénoy. The volume is well produced and is a fine record of work done.

W. B. B.

REPORT OF THE COUNCIL

OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS FOR THE YEAR, 1921–2. PRESENTED TO THE ANNUAL GENERAL MEETING, FEBRUARY 24TH, 1922.

DURING the year eight meetings have been held which have usually been devoted to discussions. The meetings have been well attended, an average of 77 members and friends being present. The following sixteen communications have been made to the Association:

Dr A. S. Horne (Imperial College of Science). "(I) Cultures of Polyopeus. (II) Demonstrations of the Enzymic action of Polyopeus in Solid Nutrient Media containing Starch."

Dr S. G. Paine (Imperial College of Science). "(I) Cultures of the Causal Organism of a Potato Disease. (II) A Novel Method of Inoculation of Potato Tubers."

Dr Wm B. Brierley (Rothamsted Experimental Station). "(I) The Differential Infection of Pure Lines of Wheat by Biological Forms of *Puccinia graminis tritici* (from the Department of Plant Pathology, University of Minnesota)."

Mr G. C. Gough (Ministry of Agriculture). "(I) Potato Tubers Infected Simultaneously by Corky Scab and Wart Disease."

Dr J. Davidson (Rothamsted Experimental Station). "The Cells of Plant Tissues in Relation to Cell Sap as the Food of Aphids."

Mr E. R. Speyer (Lea Valley Research Station). "Ceylon Ambrosia Beetles and their Relation to Problems of Plant Physiology."

Mr Millard (Leeds University). "Green Plant Matter as a Decoy for Actinomyces Scabies in the Soil."

Mr E. H. Richards (Rothamsted Experimental Station). "The Action of Bacteria and Protozoa in Conserving the Nitrogen in Sewage."

Mr Wiltshire (Long Ashton Research Station). "The Methods of Infection of the Apple Canker Fungus."

Mr Engledow (Cambridge Plant Breeding Institute). "The Problem of Increasing the Yields of Cereal Crops by Plant Breeding."

Mr Saunders (National Institute of Agricultural Botany). "Some Problems of Seed Testing."

Dr W. Brown (Imperial College of Science). "The Physiology of the Infection Process."

Dr E. J. Butler (Imperial Bureau of Mycology). "Meteorological Conditions and Disease."

Professor J. H. Priestley (Leeds University). "The Resistance of the Normal and Injured Plant Surface to the Entry of Pathogenic Organisms."

Professor Stebbing (Edinburgh University). "The Importance of Scientific Research in Forestry and its Position in the Empire."

Dr J. Rennie (Aberdeen University). "(I) The Present Position of Bee Disease Research. (II) Polyhedral Disease in Tipula Species."

A Field Meeting was held on July 14th, 1921, when the Association was entertained at Reading by Messrs Sutton and Sons and by Professor Percival and the authorities of the Reading University College.

During the year Mr E. E. Green, finding himself unable to retain his seat on the Council, his resignation was accepted with reluctance and Dr J. Waterston of the Natural History Museum was invited to fill the vacancy. Since the last Annual General Meeting Professor Johannsen of Denmark has been elected to Honorary Membership of the Association and eighteen candidates to ordinary membership. The number of members exclusive of those whose subscription is three or more years in arrears now stands at 240, an increase of nine over last year.

The Laws of the Association have been revised and the amended Laws were published in Part I of Volume IX of the *Annals of Applied Biology*. The issue of the *Annals* has been brought up to date and in future it is hoped to complete one volume each year.

The increase of the annual subscription to the Association to 25*s*., passed by resolution at the last Annual General Meeting, came into force on January 1st of this year.

The Council consider, and are confident that members will agree, that the Association may be congratulated on the progress made during the year as judged whether by the value of the communications received or by the growing membership and the remarkable and increasing attendance at the meetings.

The thanks of the Association are due to Professor J. B. Farmer and his colleagues for granting the use of rooms for the meetings of the Council, and, further, for their unfailing kindness and hospitality in permitting the Association to meet in the Botanical Lecture Theatre of the Imperial College of Science.

OBITUARY

A. W. BACOT

THE death of ARTHUR WILLIAM BACOT, Entomologist to the Lister Institute of Preventive Medicine, took place in Egypt on April 12th last. Bacot had gone out to Cairo, earlier in the year, at the request of the Egyptian Government to investigate problems connected with the aetiology of typhus fever. It is now a well known fact that this disease is transmitted from one human being to another through the agency of the body louse, and great advances have been made in its control by the energetic eradication of that objectionable insect. There are, however, still important links in the chain of evidence with regard to the exact means by which the louse is able to infect man with the disease, notwithstanding the large amount of experimental work which was undertaken during the War. The great prevalence of typhus fever in Egypt rendered it urgent that further investigations should be carried out and, when the choice fell upon Bacot to conduct researches on the entomological aspect of the problem, the Egyptian Government secured the services of an investigator uniquely qualified for the task. Mr Bacot possessed great skill as a manipulator, together with a wide experience of insect-borne diseases, and of typhus fever in particular. These qualifications were coupled with a sterling honesty of character, which led him to subject every conclusion which he formulated to the most rigid tests which he could devise. His work in consequence is of a very high scientific merit, and of quite exceptional trustworthiness.

Bacot was born in 1866, but was not educated for a scientific career, and he became a clerk with a commercial firm in the city. He remained in this capacity for a number of years, devoting his leisure to observing and rearing *Lepidoptera*, which had interested him from an early age. He was never a collector in the ordinary sense: problems relating to the bionomics and getetics of his favourite order mainly occupied his attention, and he contributed various notes and papers dealing with these aspects of his subject. Bacot eventually relinquished his office career and was appointed on the staff of the Lister Institute in 1911. His first researches in medical entomology were concerned with the bionomics of rat fleas, which were undertaken in connection with the work of the Indian Plague Commission. In 1913, in conjunction with Martin, he demonstrated that an important method of transmission of bubonic plague takes place through the medium of the proboscis of the flea. When that insect becomes gorged with the blood of an infected rat, the plague Bacillus increases to such an extent within the gut that the organism forms a mass that plugs the entrance to the stomach of the

flea, thereby blocking the passage of the alimentary canal. Further efforts made by the insect at sucking only result in blood being imbibed as far as the oesophagus, and a certain amount of the blood which is taken in flows back into the puncture. Since this blood is freely contaminated with plague bacilli an uninfected host is rendered liable in this way to contract the disease. In 1914 Bacot went out to W. Africa as a member of a Yellow Fever Commission instituted by the Colonial Office. While on this work he made a number of observations on the bionomics of the mosquito *Stegomyia fasciata*, which is the intermediary host of the disease in question. During the period of the War, Bacot was mainly engaged in investigating the body louse and its relation to trench fever. After the conclusion of hostilities he turned his attention to the rôle which that same insect performs in the transmission of typhus fever from man to man. In this capacity he did useful work in Poland. Armed with the experience thus gained, he proceeded along with his colleague Arkwright to Cairo, early in the present year, and there continued to work at aspects of the same problem in the Laboratories of the Institute of Public Health. Unfortunately both men fell victims to the disease not long after their arrival in Egypt. How Bacot became infected does not appear to be known. He was removed to the fever hospital at Abbassia, but succumbed to the malady in rather less than three weeks after the first symptoms appeared. His colleague happily survived and we understand that he is now on the road to recovery. Bacot's funeral took plaee at the British cemetery, old Cairo, and was attended by a concourse of people, both English and Egyptian, representing many branches of scientific work. His body was borne to its final resting place by friends and colleagues working in the same laboratories.

Bacot's death adds another name to the roll of investigators who have given their lives while endeavouring to solve problems connected with this virulent disease. His place is a hard one to fill, and he has left an enduring name in the annals of medical entomology. The Association of Economic Biologists loses a valuable member, and he had only been elected to the Council of that Society during the present year. Bacot became a Fellow of the Entomological Society of London in 1907, and probably many entomologists recall his last attendance at a meeting, towards the end of last year, when he exhibited microphotographs of the eggs of the European and oriental species of an insect also concerned with the transmission of disease.

A. D. IMMS.